DECONVOLUTING THE ROLES OF INDIVIDUAL DRUGS IN COMBINATION THERAPIES

by

TOBIN PAEZ

(Under the Direction of EUGENE DOUGLASS)

ABSTRACT

The discovery of cytotoxic monotherapy drugs addressed some limitations in treating cancer patients with surgery and radiation. Although promising, monotherapies had limitations of their own. During the 1960s, oncologists at the National Cancer Institute uncovered a clinical algorithm for effectively designing chemotherapy drugs in the form of the Fractional Kill Hypothesis. This working hypothesis underlies current clinical practice and modern scientific rationale for chemotherapy efficacy, stating that a combination chemotherapy response rate is the independent product of individual drug response rates. This project tests this hypothesis through creating clinical databases of response rates for historical monotherapy and modern chemotherapy regimens to assess the agreement between monotherapy and combination response rates. Results demonstrated that breast, lung, and ovarian cancer exhibited disagreement by producing underpredicted responses rates. Intriguingly, colorectal cancer showed disagreement by producing enhanced response rates to several combinations, which could reflect synergistic drug interactions for some combinations.

INDEX WORDS: Combination Chemotherapy, Monotherapy, Cytotoxic, Cancer, Independent Drug Action

DECONVOLUTING THE ROLES OF INDIVIDUAL DRUGS IN COMBINATION THERAPIES

by

TOBIN CHRISTOPHER PAEZ

BACHELOR OF SCIENCE, NORTHERN ARIZONA UNVERSITY, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2022

DECONVOLUTING THE ROLES OF INDIVIDUAL DRUGS IN COMBINATION THERAPIES

by

TOBIN CHRISTOPHER PAEZ

Major Professor:	Eugene Douglass
Committee:	Kevin Dobbin
	Mandi Murph

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
MAY 2022

ACKNOWLEDGEMENTS

I would like to thank Dr. Eugene Douglass for all his help, words, and phrases of wisdom, understanding, and guidance in navigating through this project. I would also like to thank my lab mates, Liz, Ashley, Emily, and George, for their support and comedic relief throughout the course of this program. I would also like to thank Amy Jeffries for not only being the lab mom, but for also being our "union rep". I would also like to thank my family for their long-distance encouragement throughout this program. Lastly, I would like to thank Alanna Koritzke for her unconditional love and support of my professional goals and aspiration of becoming a physician.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Chapter Overview: The first subsection will give an overview on the history of cancer and cancer treatment from ancient times through modern day. In addition, the limitations of cancer treatment modalities will be discussed. The second subsection will discuss an autobiographical novel titled *The Death of Cancer,* which describes the story of how oncologists at the NCI created the Fractional Kill Hypothesis, and a clinical algorithm for combination chemotherapy drug design. Both concepts remain relevant in modern research settings and clinical practice. The third subsection will discuss the importance of cytotoxic chemotherapy drugs on a global scale and evidence that supports the continued relevance of cytotoxic chemotherapies in cancer treatment.

Subsection 1: History of Cancer

Cancer has posed a threat to human health for as long as mankind has existed, with the earliest documented cases dating back Ancient Egypt in 3000 BC as described in the Edwin Smith's papyrus (2). Interestingly, some experts argue that cancer may have existed during prehistoric times, with evidence suggesting that cancer may affected dinosaurs (2,3). Nevertheless, it is surmised that cancer was not nearly as prevalent in ancient human civilizations as it is today due to a significantly lower average lifespan, although there are no precise statistical documentations of ancient cancer incidence rates for direct comparison. However, this argument is made largely due to advances in conceptual understanding of cancer and the correlation between cancer incidence and age, which indicates older patients are more likely to get cancer than younger patients (3).

Understanding that cancer risk increases with age explains prevention screening guidelines that are recommended for patients who are around 50 years or older, depending on the type of cancer. However, the recommended age for other cancer screenings is decreasing to include slightly younger populations. Regardless, the average lifespan of humans has drastically increased over the past 100 years or so. Per the Center of Disease Control and Prevention life expectancy data, the average lifespan for both sexes in the US during 1900 was 47.3 years and has since increased to 78.7, with the most drastic increase occurring between 1900 and 1950, which was from 47.3 to 68.2 (5). Reasons for this increase can be largely attributed to advancements in technology, scientific understanding of diseases, healthcare, and sanitary practices. This increase in age, however, introduces an increased risk of acquiring diseases such as heart disease and cancer (6). Since the average human lifespan was much shorter in ancient times as compared to today, this is one reason why cancer is largely viewed as a problem that has a more pronounced effect on humans in the modern era versus humans during ancient times. Other reasons why modern humans are more likely to be affected by cancer is due to lifestyle and environmental factors, such as exposure to tobacco and pollution (6). This is not to say that humans of ancient civilizations were not afflicted by cancer, but they were more likely to succumb to other health issues, such as infectious disease and starvation (4).

Since physicians during ancient times were limited in their ability to diagnose cancer, there were no histological confirmations of documented cases of cancer. Importantly, cancer was not microscopically illustrated until 1838 (7). Additionally, the term “cancer”, specifically described as “carcinoma” was not coined until around 400 BC by the Greek physician Hippocrates (1,4). However, early Ancient Egypt descriptions of the disease were similar to modern day gross characterizations of cancer. For example, in Edwin Smith’s papyrus in 1500BC, the presence of breast cancer was described as ulcerated lesions, which would reoccur

after treatment with cauterization and ablation techniques (1). Removal of superficial tumors, such as skin growths were easier to cure through these techniques. Still, recurrent, or deep tumors, such as breast cancer, were regarded as incurable since there were no effective treatment options (1,8).

Shifting focus to the late 18th through the 19th century, physicians notably began to explore different surgical procedures for treating patients with cancer (7,8). One major example was the introduction of radical mastectomies for breast cancer in 1774 (6). This procedure was routinely practiced and documented in the 1880s by William Halsted, who successfully incorporated aseptic and anesthetic techniques into surgery (9). This procedure, which involved extensive muscle and soft tissue excision, was revised to the current standard of radical mastectomies that was established in 1972 by John Madden. Madden's procedure aimed to preserve muscle tissue and eliminate the unnecessary excision of unaffected tissues, which provided patients with better outcomes such as improvement in quality of life (9). As more procedures were discovered, doctors began to heavily rely on a vast number of surgical procedures to remove tumors throughout the 1900s (**Fig. 1).**

In addition to surgical intervention, the discovery of radiation therapy introduced an alternative and effective option for cancer treatment. X-radiation was discovered in the late 1800s, by Professor Wilhelm Roentgen (10). This discovery was quickly translated into medical devices that could emit x-rays for diagnostic purposes. Shortly thereafter, it was discovered that in optimal doses radiation therapy could effectively treat cancer and improve patient outcomes. Different dosing regimens and intensities of radiation were used dependent on the tumor location. For example, less intense radiation was necessary for tumors that were on the skin surface versus a tumor deep within the abdominal cavity. One of the earliest pioneers in radiation therapy was Emil Grubbé, whose first use of radiotherapy was to treat a patient with breast

cancer (12,13). Although radiation therapy demonstrated curative effects, it was also determined that inappropriate dosing and exposure could increase the patients' risk for cancer. This was attributed to not fully understanding how radiation therapy affected healthy cells in addition to cancer cells (14). However, through technological advancements, radiation instruments were made that could precisely and accurately deliver desired doses of radiation to the site of interest.

During the 1960s, surgery and radiation therapy continued to be the gold standards for cancer treatment. Although these treatments were partially effective, it was evident that these modalities had significant limitations in providing adequate treatment for all tumor types.

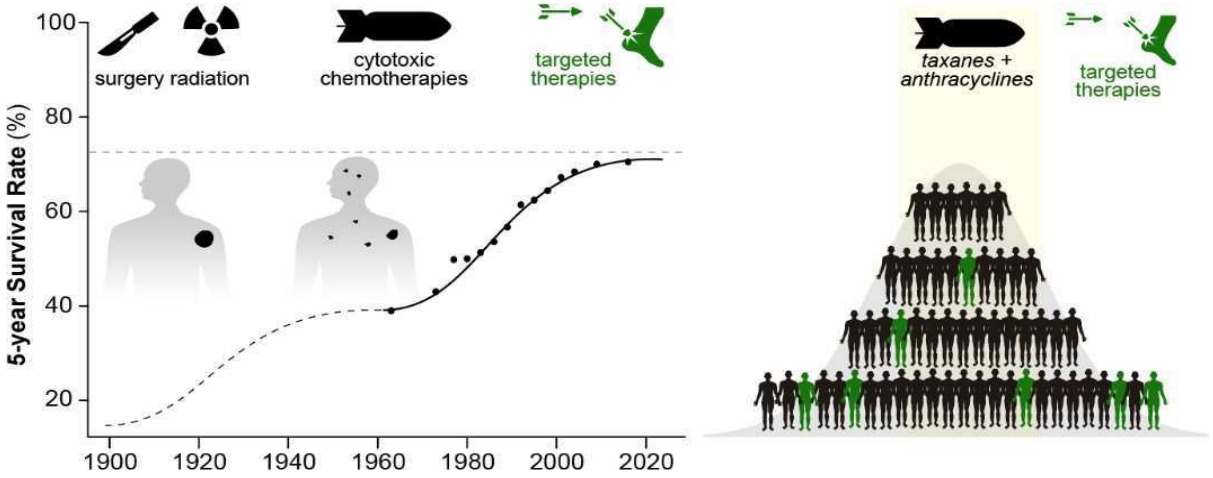


Figure 1. The graph (left) demonstrates the 5-year survival rate of all cancer patients since 1900. The dotted line represents a speculative trend of five-year survival percentages from the early 1900s-1960s due to the lack of an unofficial record system of cancer incidence. The solid line represents the 5-year survival rate trend based on the Seer Cancer Statistics published by the National Cancer Institute. This figure demonstrates that while monotherapy drugs were being used in the 1960s, survival rates were underwhelming. The bell curve (right) is a comparison of how many patients successfully respond to a combination of cytotoxic chemotherapy drugs (black) versus targeted therapies (green).

Patients with cancer would often still succumb to their illness due to the inoperable nature of the cancer or due to metastatic capabilities of tumors. While surgery may be able to remove the primary tumor, surgery is unable to remove all microtumors or metastases that have spread to different organ systems. Additionally, treating metastases or liquid cancers with radiation therapy was also viewed as futile due to similar limitations. Although 5-year survival rates began to increase due to surgery and radiation advancements, these survival rates would eventually hit

a plateau in the 1960s (15) (**Fig. 1)**. Patients who were unfit for surgery or radiation had no other viable treatment options, and therefore were subjected to palliative care throughout the remainder of their terminal illness.

Although surgery and radiation were the standards of care in the 1960s, efforts to discover new cancer treatments began in 1940s. The first single agent cytotoxic chemotherapy drug created was nitrogen mustard, which was used to treat lymphoma. This drug was derived by scientists who were studying compounds related to mustard gas to create better chemical warfare agents during World War II (16). Shortly after, other scientists began to follow suit and discover compounds effective for a variety of cancers. One notable study, as conducted by Dr. Sidney Farber in 1948, demonstrated the first successful cancer chemotherapy in treating children who had Acute Lymphocytic Leukemia (ALL). Dr. Farber demonstrated that treatment with aminopterin, a folic acid derivative, and methotrexate, an antimetabolic cytotoxic agent, could produce positive results in children with leukemia (17). The discovery of these cytotoxic chemotherapies had provided physicians with a possible solution for treating patients with cancer. The potential for success in treating patients with chemotherapy was demonstrated by the first cure of metastatic choriocarcinoma, which occurred in 1956 (18).

When compared to surgery and radiation therapy, cytotoxic chemotherapy demonstrated potential for significant improvement in patient outcome during the 1960s (**Fig. 1**). Although single agent cytotoxic chemotherapies provided physicians and patients with a new treatment modality, it was not without significant limitations in successful patient outcomes. While few patients would respond relatively well to treatment, many others would remain unresponsive or experience poor outcomes due to tumor resistance to the drug (**Fig. 2A**).

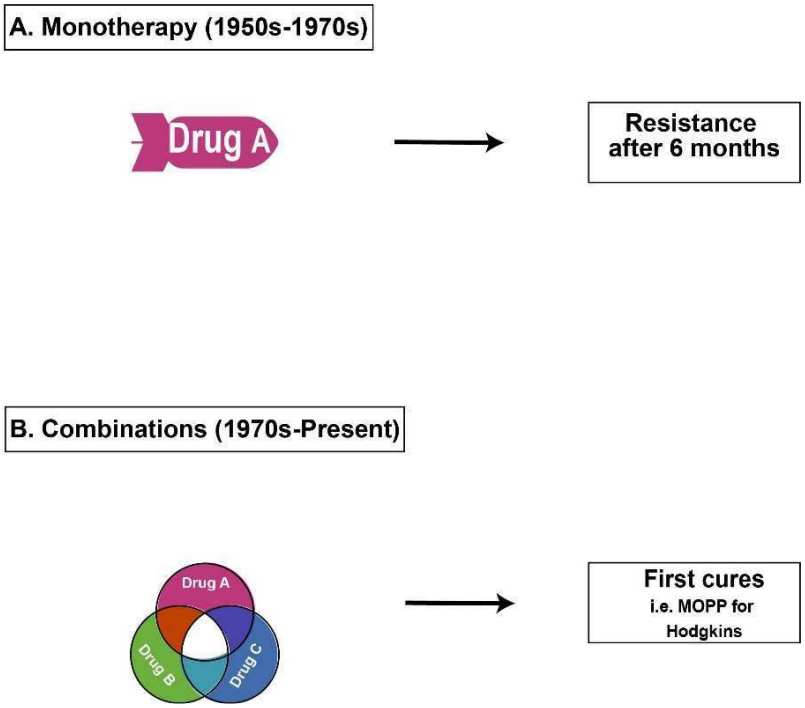


Figure 2. A. This demonstrates that single agents, Drug A, often produced temporary improvement patients, which was a significant limitation during the early stages of chemotherapy treatment. B. Once combination chemotherapies were discovered and became a widely acceptable form of treatment, patient outcomes began to significantly improve.

Physicians and scientists began to investigate reasons why patients responded poorly to treatment and experimented with ways in which these issues could be overcome. The most impactful discovery made during these investigations was the concept of combining single agent cytotoxic chemotherapies into a combination treatment, which increased in popularity in the 1970s (**Fig. 2B)**.

The concept of combination chemotherapies was inspired by a groundbreaking discovery of treating tuberculosis in 1950. Scientists demonstrated that tuberculosis could be cured by combining two antimicrobial agents and administering both in a single dose (19). The success demonstrated by this finding lead oncologists and scientists to experiment with the notion that combination chemotherapy may be able overcome the limitations seen in the clinic. In 1952, a famous study conducted at the National Cancer Institute (NCI) demonstrated promising

outcomes when combining antileukemic drugs in treating mice with Acute Lymphocytic Leukemia (20). Shortly after, other pioneering oncologists at the NCI began their investigation into treating patients with combination chemotherapies. This early evidence showed promise in treating cancer with chemotherapy, which led to the funding of an official drug screening center at the National Cancer Institute in 1955 (21).

Subsection 2: Death of Cancer

One of the cornerstones for my research project stems from the autobiographical novel called *The Death of Cancer*, which was written by Dr. Vincent T DeVita (22). In this book, Dr. DeVita recalls his journey as physician at the NCI. He originally began as a new graduate clinical associate within the oncology ward in 1963. After holding several other positions throughout his journey, Dr. DeVita finished his career as the Director of the NCI and National Cancer Program. Dr. DeVita shared his experiences in treating cancer patients with the standards of care: surgery and radiation therapy. Although, by this time a handful of monotherapy drugs had been discovered, cancer research continued to remain an uncharted frontier due to ethical concerns surrounding toxicity and patient harm. However, the grim outcomes and disposition of his patients inspired Dr. DeVita and his team of oncologists and scientists to look past the opposition and continue to investigate cytotoxic chemotherapy drugs with the hope of developing more effective treatments (22).

Despite the strong opposition from others within in the field, Dr. DeVita and his colleagues continued to investigate better chemotherapy regimens. During this exploration, the oncologists at the NIH established several key concepts for success chemotherapy treatments. Dr. Skipper, a colleague of DeVita's, surmised that in order to cure a cancer, every tumor cell needed to be killed. They postulated that it was not sufficient to kill only 99% of cancer cells, because the remaining 1% of cancer cells could be enough for the tumor to regrow and kill the

patient due to insufficient tumor response to treatment. This concept was proven experimentally by the oncologists and scientists at the NCI using mouse models (22). Additionally, the oncologists established the Inverse Rule, which essentially stated that the more cancer cells that exist at the start of a treatment, the less likely the cancer could be cured. In other words, this stated that certain cancers were less likely to respond to single agent cytotoxic chemotherapy (22).

One of the most impactful contributions of the oncologists at the NCI was the formation of the Fractional Kill Hypothesis. This working hypothesis, which remains relevant today, explains that cancer drugs kill a constant fraction of cells per dose, not a fixed number, where the fraction is independent of the number of pre-existing cancer cells. If cytotoxic chemotherapies killed a fixed number of cells, creating a cure for cancer would be easier to achieve (22).

The reason why tumor cells are killed in this manner is due to cell heterogeneity within a single tumor. Individual cancer cells may not all exhibit the same mutations that result in tumorigenesis. As a result, a single tumor will not be entirely responsive to a single antitumor drug (**Fig. 3).**

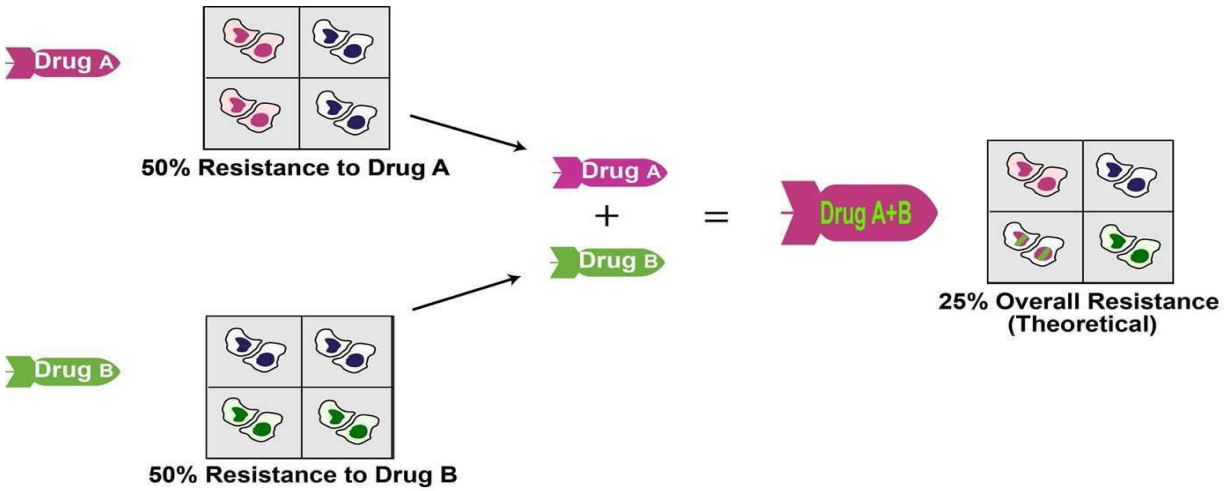


Figure 3. This diagram represents conceptual framework for the Fractional Kill Hypothesis, a premise established by the oncologists at the NCI during the 1960s. If 50% of all tumor cells are resistant to Drug A, and if the 50% of same tumor cell population is resistant to Drug B (with overlapping cellular response to both drugs), then through combining Drug A + Drug B it is expected that only 25% of the total tumor cell population exhibits drug resistance.

For example, some cells within a tumor are only responsive to an alkylating agent such as cyclophosphamide, whereas other cells are only responsive to anthracycline drugs such as doxorubicin. This is due to the differing mechanisms of actions of the two drugs. Alkylating drugs act to inhibit cell growth through the addition of alkyl groups to nitrogen bases. This alters a cell's ability to properly transcribe DNA into RNA for protein synthesis, resulting in apoptosis (23). In contrast, the mechanism of action for anthracycline drugs such as Doxorubicin, which is an antibiotic agent, slows cell growth by inhibiting the function of topoisomerase II, which is a key enzyme involved in formation of functional DNA (24). By combining chemotherapies with different mechanisms of action, the underlying assumption of the Fractional Kill Hypothesis is that the patient's tumor has a statistically higher likelihood of responding to one of the two drugs in a given combination.

With the establishment of the Fractional Kill Hypothesis, the oncologists began to design combination chemotherapy drugs. Throughout this exploration, they determined three overarching criteria must be satisfied for successful combination drug design (**Fig. 4**). As a result of their observations, oncologists at the NIH believed that the most fundamental requirement was combining drugs with independent mechanisms of action. Dr. Skipper was able to successfully formulate a combination of drugs with intricate dosing schedules that cured mice with ALL (25). Several years later, Dr. DeVita created one of the first successful combination chemotherapies for Hodgkin's disease. This treatment, known as MOPP, included Mustargen (an alkylating agent), Oncovin (a vinca alkaloid), Procarbazine (an alkylating agent), and Prednisone (a corticosteroid) (26). Through observations

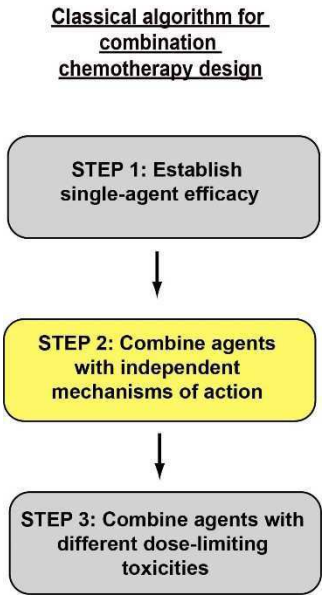


Figure 4. This algorithm demonstrates the three single-agent requirements for combination design as discovered by scientists and the NCI oncologists during the 1950s-1960s.

when treating patients with MOPP, Dr. DeVita and his colleagues reaffirmed the idea that there must be zero cancer cells at the end of a treatment regimen to obtain successful patient outcomes. Any remaining cancer cells can replicate causing significant problems for patient outcomes. As a result of their relatively successful investigations, the clinical design for combination chemotherapy follows the same algorithm decades later (26). The discovery of combination chemotherapy provided significantly improved treatment options for cancer patients who were nonresponsive to surgery, radiation, or monotherapy.

The mounting evidence that supported combination cytotoxic chemotherapy as a legitimate form of cancer treatment led to the official declaration of war on cancer. In 1971, President Richard Nixon signed the National Cancer Act, which enabled the NCI to establish a National Cancer Program to promote extensive research on cancer through cancer research centers and database systems (27). The increased federal support and funding of cancer research led to a decrease in the incidence and mortality rates, and an increase in the 5-year survival rate for all cancers by the 1990s (22,28). These improving trends can be greatly attributed to the efforts of the ambitious and trailblazing oncologists at the NIH during the early 1950s and 1960s as shown in **Figure 1**. The importance of cytotoxic chemotherapy drugs remains relevant today.

Subsection 3: Current Clinical Relevance of Cytotoxic Chemotherapy

Although cytotoxic combination chemotherapies provided a new sense of optimism for cancer treatments, this modality also experienced similar limitations as monotherapy drugs in terms of resistance. As with surgery and radiation, patient survival rates when treated monotherapy drugs resulted in an underwhelming response. Consequently, the paradigm of cancer research and clinical practice shifted towards precision medicine in the form of targeted therapies and immunotherapies, through the utilization of genomic sequencing (29). Examples of

these therapies include growth signal inhibitors and angiogenesis inhibitors, which work to control the cell cycle and the dispersion of cancer cells by acting on a specific molecular target (30). An example of a commonly targeted site is the Human Epidermal growth factor Receptor 2 protein (HER-2) in patients with a specific subtype of breast cancer. If the patient's tumor histologically overexpresses the HER-2 receptor then the patient qualifies for treatment with a specific targeted therapy drug, such as trastuzumab. Treatment with this agent alone, or in combination with other drugs, has been shown to be effective in treating HER-2 positive breast cancer patients (31).

Although there has been success with targeted therapies, one limitation is that targeted therapies only work for a small number of patients due to the tumor-specific nature of the drug. Referring to the previous example, if a breast cancer tumor does not overexpress the HER-2 receptor, then patients will not receive trastuzumab since it will be ineffective against their tumor type. As a result, patient tumors must be characterized prior to treatment through molecular testing, to determine what type of treatment will be effective for their tumor. In contrast with targeted therapies, cytotoxic chemotherapies cover a broader range of tumor types within a given patient population (**Fig. 1**). This is due to cytotoxic chemotherapies working by targeting all cells in the body, including healthy noncancerous cells (31,32).

Another limitation with targeted therapies is that these innovative treatments are often very expensive, which can cause financial limitations for patients with lower incomes (32). In 2021, a study was published in The Lancet Oncology by a group of investigators who conducted a global study to determine which antitumor drugs were considered the most essential (33). A questionnaire containing 28 questions was sent to practicing oncologists from across 89 countries of varying levels of overall income. The principal question, however, was for responders to designate which 10 antitumor drugs would make the most significant impact on public health

within their respective country. At the time of analysis, there were responses from 948 participants across 82 countries. Approximately 82% of the respondents were from upper-middle and high-income countries, with the remaining 18 % coming from low and lower-middle income countries (33). While the results slightly differed based on the income level of the countries, the overall top three most chosen drugs were all cytotoxic chemotherapies: Doxorubicin, Cisplatin and Paclitaxel. In low-income and lower-middle income countries, 8 of the top 10 drugs included cytotoxic drugs, one response included Tamoxifen which is an estrogen modulator, and one response was Imatinib which is a targeted cancer drug for treating leukemia (33).

This study provided an invaluable insight into how cytotoxic chemotherapies continue to remain highly relevant, regardless of a country's average income level. Additionally, this study demonstrated that, while the paradigm of cancer treatment is shifting toward targeted therapy, much of the world continues to rely on cytotoxic chemotherapy (33). Cytotoxic chemotherapies are especially important in developing countries that do not have the same access to biotechnological or financial resources as wealthier and more developed countries. It is important to note that even in high income countries, only 5 of the top 20 cancer drugs chosen were targeted therapy drugs. Of the top drugs chosen by these oncologists, 19 out of 20 (95%) are included on the World Health Organization's Essential Medicine List, which means that there is continued agreement that cytotoxic chemotherapy remains an essential standard of care for cancer globally (33,35).

Another financial burden of targeted therapies is the genomic testing that occurs prior to treatment. In addition to the price of the actual drugs, which could range from hundreds to hundreds of thousands of dollars, patients are also responsible for the costs related to the molecular profiling their cancer (36). The price of these tests can range anywhere from several hundred to several thousands of dollars for a single test. Additionally, several of these tests,

which may continue to be under patent may remain widely unavailable in parts of the world due to financial, technological, and healthcare system constraints (37, 38). Many of the currently relevant drugs have been around for several decades. As a result, these drugs are no longer patented, and, in combination with technological innovations, they can be relatively cheaper and easier to mass produce. This would allow for cytotoxic chemotherapies to be accessible in many countries with lower-than-average incomes (33,39). Newly developed targeted therapies, however, can remain under US patent for at least twenty years, which means that generic biosimilar drugs are not available for two decades (34). Several of the cytotoxic drugs listed in The Lancet study have been around for decades, and as a result, these drugs are no longer under patent and are significantly more affordable (33). As such, cytotoxic chemotherapies continue to have clinical relevance today, thus warranting further research and understanding.

While the breakthrough discovery of combination chemotherapies at the NCI gave hope for cancer patients, the underlying mechanisms to explain these clinical outcomes are still being elucidated. The Fractional Kill Hypothesis describes patient outcomes as the result of individual cancer cell response to a drug. Additionally, this hypothesis is built on the assumption that drug action remains entirely independent within a combination. This means that a combination of drugs do not work together in a synergistic fashion to provide any added benefit. In more recent literature, scientists such as Dr. Peter Sorger at Harvard Medical School aim to investigate and validate these concepts. The goal of this project is to use clinical trial data to test these hypotheses established by the pioneering oncologists at the NCI and by Dr. Sorger.

CHAPTER 2

BACKGROUND

Chapter Overview: This chapter aims to discuss clinical and mathematical concepts that are applied in this project. The first subsection will discuss different clinical outcomes measured in cancer clinical trials, such as overall survival and objective response rate. The second subsection will discuss how weighted averages were used in this project to generate a final clinical response rate for each drug-cancer pair. The third subsection will discuss how joint independent probability relates to the Fractional Kill Hypothesis. The final subsection will discuss how the Fractional Kill Hypothesis underlies to current research rationale by discussing Dr. Peter Sorger's work.

Subsection One: Defining Clinical Outcomes

Clinical outcomes are determined by several different endpoints, which are used to measure the benefits of the treatment under investigation. For new or existing cancer drugs to be approved for therapeutic use, the FDA requires that certain endpoints are documented when conducting clinical studies. Having data on these endpoints supports or disputes the overall efficacy and safety of the new therapeutic. The FDA provides a guidance document with recommended endpoints that should be used to assess clinical benefit of new treatments. For the purposes of this project, these endpoints will be used in the context of cancer clinical trials. While clinical observations demonstrated that combination chemotherapies significantly improved patient outcomes, additional investigation is needed to fully elucidate how these improved outcomes are achieved. By gaining a better understanding, more effective combination

chemotherapies can be established. Two commonly used endpoints, Overall Survival (OS) and Objective Response Rate (ORR), will be discussed below. (40)

OS measures the duration in which a cancer patient lives, beginning at the start of the clinical trial and ending when they succumb to their disease. This objectively measured endpoint provides reliable information about how a new cancer treatment can extend a patient's life and provide a general understanding of the drug's clinical benefit. Therefore, OS measures the benefit of a cancer drug on a long-term scale. ORR quantitatively measures the reduction in tumor size to see if the drug was effective (40). Thus, in contrast with OS, ORR focuses on the benefit of a cancer drug on a short-term scale by focusing on more immediate results. Because these endpoints are not perfectly correlated, the preferred endpoint in clinical trials is OS. However, this project focuses on ORR because of it being a drug-centric value and due to its applicability to the Fractional Kill Hypothesis. As such, the only endpoint documented was ORR, exclusively analyzing Complete Responses (CR) and Partial Responses (PR). Therefore, ORR will be discussed in much greater detail below.

ORRs measure the directly visible response of a patient's tumor to antitumor agents (39). For the past several decades, ORRs have been measured using criteria established by the World Health Organization (WHO) which was established in 1979, or more recently, by using the Response Evaluation Criteria in Solid Tumors (RECIST) established in 2000. These guidelines enable observers to collect data in a harmonized fashion. Tumor measurements are collected using medical imaging devices, such as computed tomography (CT) (39). ORR allows for a standardized collection and analysis of tumor responses within a given clinical trial. The establishment of this standard aimed to enable data comparison between different studies to establish which drugs produce the best patient response (40).

Prior to the establishment of WHO response criteria in 1979, studies used an unstandardized, therefore, inconsistent descriptions of what constituted as an ORR (42). This led to several challenges when comparing results between similar studies. After being widely accepted in the early 1980s, the WHO criteria were popular for a few decades until the development of the RECIST Version 1.0 (43). RECIST aimed to resolve several of the criticisms surrounding WHO ORR criteria, which often resulted from interobserver variability (44). This also raised speculation regarding the comparability of results between clinical trials conducted at different institutions (45). To address these needs, the RECIST criteria was created by the National Cancer Institute (NCI) of the United States, The NCI of Canada Clinical Trials group, and the European Organization for Research and Treatment of Cancer (43). In 2009, revisions were made to the original RECIST criteria. Like WHO criteria, the first rendition of RECIST criteria drew criticism, and investigators began to question the total number of lesions that were measured in a single organ. Currently, the most updated form of RECIST criteria is Version 1.1, in which the number of lesions measured for an organ was reduced from 5 to 2 (46). In this research project, clinical trials that used standardized and comparable forms of ORR criteria were included in analysis. Studies that did not explicitly use an established ORR criteria were excluded.

WHO criteria assess response to treatment based on four quantitative endpoints, which will be discussed in no specific order. WHO criteria measures all tumor lesions, and tumor size is obtained by adding the products of the two longest bidimensional diameters. The first endpoint is a CR, which is achieved by confirming that all visible tumor has disappeared after 4 weeks of treatment. The second endpoint is a PR, which is defined as the reduction of approximately 50% in tumor size after 4 weeks of treatment. The third endpoint is progressive disease (PD), which is defined as a greater than 25% increase in tumor size with no CR, PR, or

Stable Disease (SD) prior to this increase, appearance of new lesion, or the increase of one lesion by 25%. The fourth endpoint, SD, is defined as a tumor response that does not meet PR or PD criteria (42).

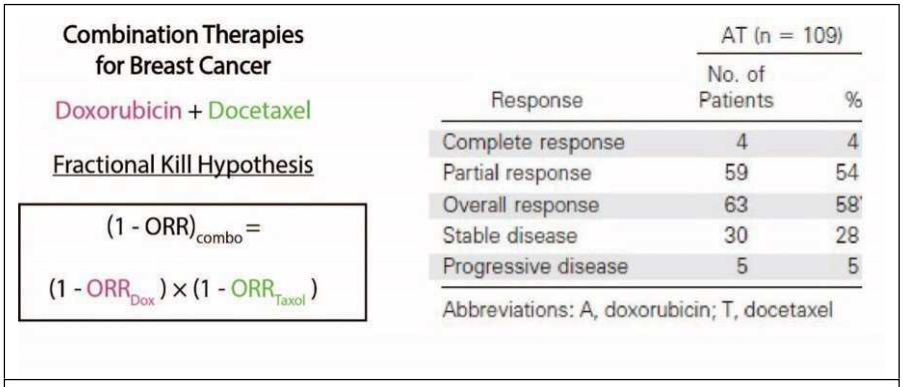
RECIST 1v.0 criteria assess response to treatment based on four quantitative endpoints, which will be discussed in no specific order. RECIST v1.0 measures 5 maximum target lesions per organ, and 10 in total. Tumor size is obtained through the sum of the longest unidimensional diameters of the target lesions. The first endpoint is a CR, which is achieved by confirming that all visible tumor has disappeared after 4 weeks of treatment The RECIST criteria defines a PR as the reduction of approximately 30% of tumor size after 4 weeks of treatment The third endpoint is progressive disease (PD), which is defined as a greater than 25% increase in tumor measurement with no CR, PR, or Stable Disease (SD) prior to increase, appearance of new lesion, or the increase of one lesion by 20%. The fourth endpoint, SD, is defined as a tumor response that does not meet PR or PD criteria (43).

RECIST 1v.1 criteria assess response to treatment based on four quantitative endpoints, which will be discussed in no specific order. RECIST v1.1 measures 2 maximum target lesions per organ, and 5 in total. Tumor size is obtained through the sum of the longest unidimensional diameters of the non-nodal target lesions and the short axis of nodal lesions. The first endpoint is a CR, which is achieved by confirming that all visible tumor has disappeared after 4 weeks of treatment The RECIST criteria defines a PR as the reduction of approximately 30% of tumor size after 4 weeks of treatment The third endpoint is progressive disease (PD), which is defined as a greater than 20% increase in tumor measurement with no CR, PR, or Stable Disease (SD) prior to increase, with this increase being at least 5mm in sum, or appearance of new lesions. The fourth endpoint, SD, is defined as a tumor response that does not meet PR or PD criteria (44).

Studies that used either WHO or RECIST to calculate ORRs were both included because of the comparable nature of the two criteria when measuring objective response rates. Although WHO and RECIST have seemingly different definitions for PR, studies have shown that these values are comparable (47,48,49). This is because mathematically, the reduction of unidimensional products by 30% is comparable to the 50% reduction of bi-dimensional products since both values equate to a 65% reduction in overall tumor volume. Since the partial response thresholds are comparable, ORR data from clinical trials using either of these criteria should be as well. It is important to note that the difference in collecting tumor measurements for WHO and RECIST does result in contrasting values for determining progressive disease (47). However, this difference does not impact the results of this research project since the primary focus is on CR and PRs, and not PD.

Subsection 2: Weighted Averages

One of the goals of this research project is to create tumor-specific datasets and analyze the ORRs of combination chemotherapies for 5 different types of cancer. To conduct this analysis, a comparison was made between two values: theoretical ORRs and clinical ORRs. Theoretical ORRs are the expected response rates for a combination chemotherapy. This expected response rate is calculated using monotherapy data.



Response	AT (n = 109) No. of Patients	%
Complete response	4	4
Partial response	59	54
Overall response	63	58
Stable disease	30	28
Progressive disease	5	5

Abbreviations: A, doxorubicin; T, docetaxel

Figure 5. The equation (left) is the underlying mathematical principle of the Fractional Kill Hypothesis. The table (right) is an example of clinical response rate data curated for this project (Source: DOI: 10.1200/JCO.2005.06.236).

For example, if two individual drugs, Drug A and Drug B, both have response rates of 50%, an expected response rate can be calculated using joint independent probability.

The equation in **Figure 5** demonstrates how a theoretical response rate value can be generated for a combination (AT) of Doxorubicin (A) and Docetaxel (T). This theoretical value can be compared to actual clinical response rates when treating metastatic breast cancer patients with AT. Based on our monotherapy values, the theoretical response rate for treating patients with metastatic breast cancer with AT is 74%. Patient response rates to treatment in the trial in this example was 63%.

For the above example, the clinical reality of treating metastatic breast cancer with AT falls short of the expected ORR value by 11%. If the hypothesis of independent action were to be assumed for all drug combinations, then it would be expected that the clinical response rates would demonstrate agreement with the theoretical response rate. That is, if there is no mechanistic interaction, whether it is in a synergistical or antagonistic manner, or overlapping resistance of the two drugs, then a significant discrepancy between the two values would not be expected. In this example, a discrepancy of 11% between the clinical and the theoretical response rates warrants investigation into these underpredicted values.

$$\frac{R_{total}}{T_{total}} = \frac{R_1 + R_2 + R_3 \ldots}{T_1 + T_2 + T_3 \ldots}$$

$$\frac{R_{total}}{\sum T} = \frac{R_1}{\sum T} + \frac{R_2}{\sum T} + \frac{R_3}{\sum T} \ldots$$

$$\frac{R_{total}}{\sum T} = \frac{T_1}{\sum T}\frac{R_1}{T_1} + \frac{T_2}{\sum T}\frac{R_2}{T_2} + \frac{T_3}{\sum T}\frac{R_3}{T_3} \ldots$$

$$\frac{R_{total}}{\sum T} = \frac{T_1}{\sum T}O_1 + \frac{T_2}{\sum T}O_2 + \frac{T_3}{\sum T}O_3 \ldots$$

Figure 6. This represents the mathematical process for creating weighted averages for each clinical trial. R is the number of responders for a trial. T is the number of evaluable patients for a trial. R_{total} is the total number of responders for a single trial. T_{total} is the total number of evaluable patients for a single trial. ∑T represents the sum of all evaluable patients for the corresponding drug-cancer pairing. O is the objective response rate.

The patient cohort size for each trial was weighted against the total number of patients for that drug-cancer pairing. The weighted value for each trial was multiplied by the corresponding O value, and all these products were added together to generate a weighted clinical response rate.

To account for all clinical trials for a given drug-cancer pairing (i.e., treating metastatic breast cancer patients with AT), a final clinical ORR value was generated by using

the weighted average of all ORRs datapoints for that specific pairing (**Fig. 6**)**.** As an example of calculating weighted averages, there were 7 analyzable clinical trials for breast cancer patients who received AT. There was a total of 651 patients enrolled in these 7 studies, which had varying enrollment sizes that ranged from 42 to 214 patients.

Additionally, the ORR values for each individual trial also varied, ranging from 17% to 69%. To normalize the data, weighted averages for individual trials were calculated with respect to patient cohort size. Once individual weighted average values were calculated, a final (total) weighted ORR value was generated by adding all individual weighted average values. In the case of the combination of AT, the final weighted ORR value was calculated to be 0.48, which was used as the final clinical response rate for the AT combination for treating breast cancer. This method of using weighted averages was used to generate a final weighted ORR value for single agent drugs in the monotherapy database. In turn, these values were used to calculate the theoretical response rate using the Fractional Kill Hypothesis equation in **Figure 5**. The final clinical response rate when treating patients with metastatic breast cancer with AT is 54%, which is 20% lower than the theoretical response rate of 74%. ORRs were entered into two separate databases, a monotherapy database, and a combination database, to generate reliable theoretical ORRs and clinical ORRs to complete this analysis. The curation processes of each database are described in Chapter 3: Database Building.

Subsection 3: Joint Independent Probability

The goal of comparing theoretical response rates and clinical response rates is to test the previously introduced Fractional Kill Hypothesis. As previously discussed, this hypothesis describes the action of antitumor drugs as killing a fixed ratio of tumor cells in a single dose, regardless of the number of tumor cells present. The oncologists at the NCI during the 1960s

surmised that effective combination chemotherapies required individual drugs to independent mechanisms of cell killing (Ref. **Fig. 3**) (22). The mathematical justification for this assumption can be based on the statistical concept of joint independent probability (50). Joint Independent Probability states that two or more events are considered independent if the outcome of one event does not influence the outcome of another. The simplest way to demonstrate this concept is by flipping a coin. If a coin is flipped three times, the probability of getting heads on the third flip is independent of the outcomes of the first two flips. Meaning, if a coin lands on tails in the first two attempts, this will not affect the outcome of the third attempt. In the case of combination chemotherapy design, the oncologist at the NCI found that if two drugs are combined, the expected results are independent of individual drug action. That is, the mechanism of one drug does not impact results of the other drug because there is no interaction between the two drugs. Another assumption made is that the drugs do not exhibit co-resistant mechanisms or overlapping resistances.

Subsection 4: Independent Action and Current Working Hypotheses

This previous notion of independent action has been largely investigated by Dr. Peter Sorger, a renowned professor of Systems Biology at Harvard Medical School, and his colleagues (50-52). Independent action is pharmacologically defined as two or more drugs that result in a greater tumor response than a single drug because the tumor is more responsive to only one of the two drugs. Therefore, the drugs do not interact mechanistically to produce a significantly greater response, which would have demonstrated a synergistic or additive relationship. In contrast to the pioneering oncologists at the NCI, Sorger also explains patient response combination chemotherapy efficacy is the result of patient-to-patient heterogeneity and that combinations work without additivity or synergy (51,52) **(Fig. 7)**. Patient benefits occur because the patient responds to one of the two d rugs and does not receive any additional benefit from the

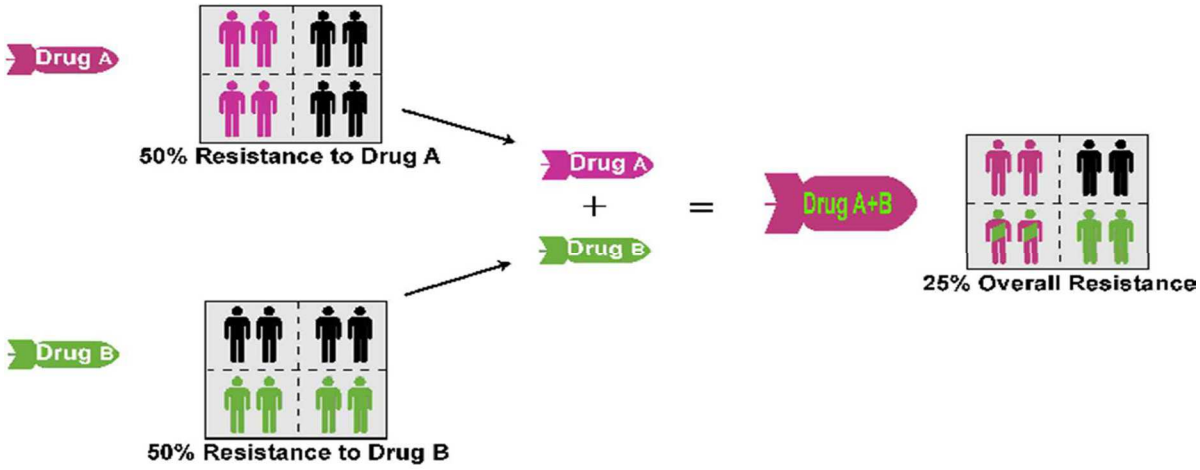


Figure 7. This figure represents the underlying principle of Dr. Sorger's hypothesis. Unlike the Fractional Kill Hypothesis, Dr. Sorger argues that individual patient response to a single drug is variable, and this variation explains the clinical benefit of combination chemotherapies.

other drug in the combination. The improved response can be attributed to combining mechanistically different chemotherapy drugs to increase the chances of the tumor responding to at least one of the drugs in the combination. Dr. Sorger explains this concept as "bet-hedging", which states that a patient has an increased chance of responding to one drug within a combination. This concept of bet-hedging, along with independent action, is sufficient in explaining substantially improved patient response rates with combination chemotherapies (51,52). For example, a combination was designed using two drugs, Drug A and Drug B, to treat a cancer patient population (**Fig. 6)**. Within the patient population, 50% of patients respond to Drug A. Within the same population, 50% of patients respond to Drug B. However, there is overlapping patient response in which 25% of patients respond to both the single agents, when administered separately. By combining these two single agents, patients have a statistically higher chance of responding to one of the two, or both drugs, which is illustrated by a decrease in overall resistance to 25%. This variability in patient response to single agents is the premise of Dr. Sorger's working hypothesis. Dr. Sorger findings demonstrate that combination chemotherapy drugs do not confer synergistic or additive effects. The goals of my research

project are to explore Dr. Sorger's findings, along with the Fractional Kill Hypothesis, through the utilization of monotherapy and combination chemotherapy response rates.

CHAPTER 3

MATERIALS AND METHODS

Chapter Overview: This chapter will discuss the data curation processes for the monotherapy and combination therapy databases. Subsection one will discuss how the foundational clinical data for the monotherapy database was curated from a textbook released by the NCI in 1970 called the *Single Agents in Cancer Chemotherapy*. Subsection two will discuss how the total amount of clinical data in the monotherapy database influenced which cancers would be explored in this project. Subsection three will discuss how the combination chemotherapy database was built by utilizing the reputable cancer clinical trial data repository called HemOnc.org.

Subsection 1: Monotherapy Database

As discussed in Chapter 2, a final clinical response rate is calculated using weighted averages calculated using all the combination chemotherapy ORR data for each drug-cancer pairing. To account for the varying cohort sizes of individual trials, the total number of patients in each trial was accounted for in the weighted average. Theoretical response rates were calculated using the same method, but instead values from monotherapy clinical trials were used.

To generate theoretical response rates for this project, our research team collaborated to build the monotherapy database. The clinical studies used for this database were largely curated from a textbook of clinical data released by the National Cancer Institute in 1970. The textbook, titled *Single Agents in Cancer Chemotherapy*, contains 780 clinical trials conducted from 1955-1970. This textbook contained data on 19,958 patients for 19 cancer drugs across 19 cancer types. The authors, Dr. Robert Livingston, and Dr. Stephen Carter, included important information for each antitumor drug such as mechanism of action, toxicity issues, dosing regimens, number of patients evaluated, number of patients who responded to treatment, and

comments about the study (53).

At the time of these studies, standardized objective response rate criteria, such as WHO criteria had not been established. As a result, the authors made efforts to standardize the data by only including studies that defined objective responses as a tumor reduction of greater than 50% in measurable lesions. The authors' main intent in publishing this book was to help bridge gaps in the understanding of single agent chemotherapy drugs during the 1970s. The authors were able to provide foundational information on how some of the most common cancers respond to monotherapy drugs. Ultimately, most of results from this book demonstrated the need for investigation into combination chemotherapy (53).

The *Single Agents in Cancer Chemotherapy* textbook contains a wealth of knowledge that was previously undisclosed by the NCI. At the time, the authors admittingly recognized that there is a plethora of monotherapy clinical trial data that remains to be unpublished. This widely unavailable data provides challenges in obtaining critically important knowledge about single agents. Through their herculean efforts, the authors published this textbook with the intent of accomplishing three main goals. The first goal was to provide physicians with clinically relevant information when deciding which drug to treat patients with. The second goal was to catalogue as much clinical information and data about each individual drug. The final goal of this textbook was to provide investigators with curated information to aid in the process of designing safe and effective combination chemotherapies. At the time of this textbook's release, the authors strongly believed it was important for the trend in cancer treatment shift away from using monotherapy drugs and begin focusing on using combination chemotherapies (53).

The information contained within the NCI textbook was curated and entered into Microsoft Excel spreadsheets for each cancer and the associated drug (**Fig. 8).**

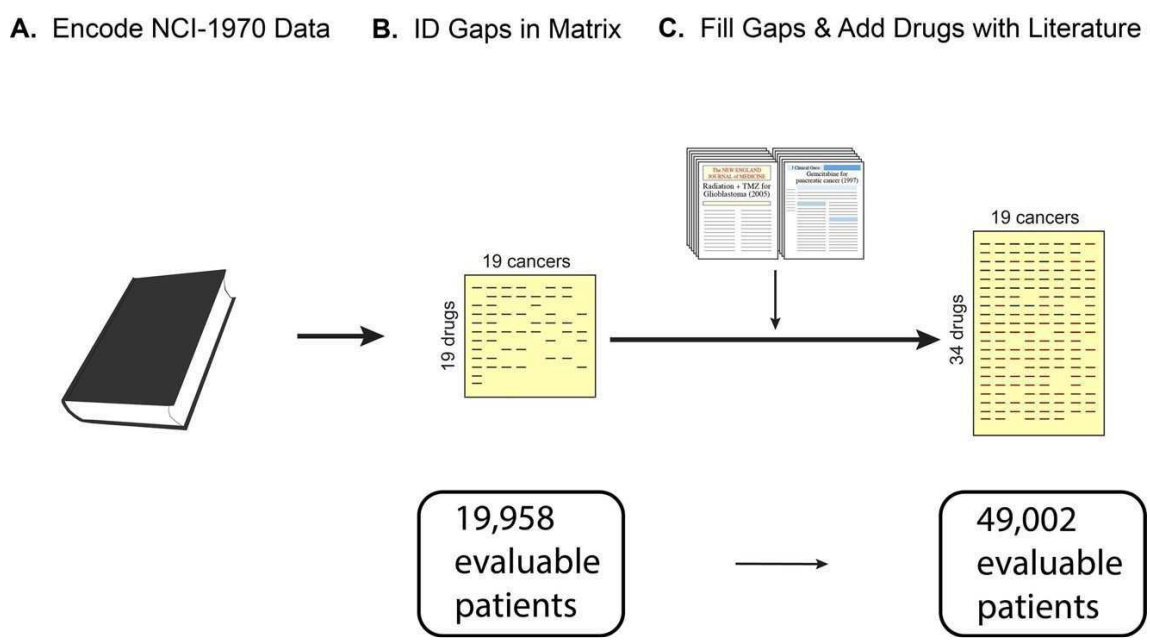


Figure 8. A. The foundation of monotherapy database was built on encoding clinical trial data from the *Single Agents in Cancer Chemotherapy*. **B.** Gaps in available patient data for specific drug-cancer pairings were identified. **C.** Over time gaps were filled with clinical data curated from clinical literature, increasing the total number of evaluable patients by 29,044.

Although this resource contained a wealth invaluable clinical trial data, it is important to note that this book is not entirely comprehensive, which became evident through identifiable gaps in response data. Over time, however, these gaps in knowledge were filled by utilizing reputable academic search engines such as Google Scholar. The papers used to fill these gaps were curated from credible journals such as the New England Journal of Medicine and The Journal of Clinical Oncology. Additionally, one of the authors of this NCI Textbook, Dr. Carter, published a series of metareviews that contained valuable monotherapy clinical trial data. As a result of these curation efforts, the monotherapy database has expanded from 19 cancer drugs to 34.

Subsection 2: Cancers Chosen for this Project

Cancers were chosen based off two main assessments: availability of monotherapy data and incidence rates (**Fig. 9.**). The availability of monotherapy was one of the driving factors since

theoretical response rates were calculated by using monotherapy rates. If monotherapy rates were not available, then the comparison theoretical and clinical response rates could not be made. Additionally, it was also important that there was a substantial amount of monotherapy available for generating representative theoretical response rates. For example, it is statistically more

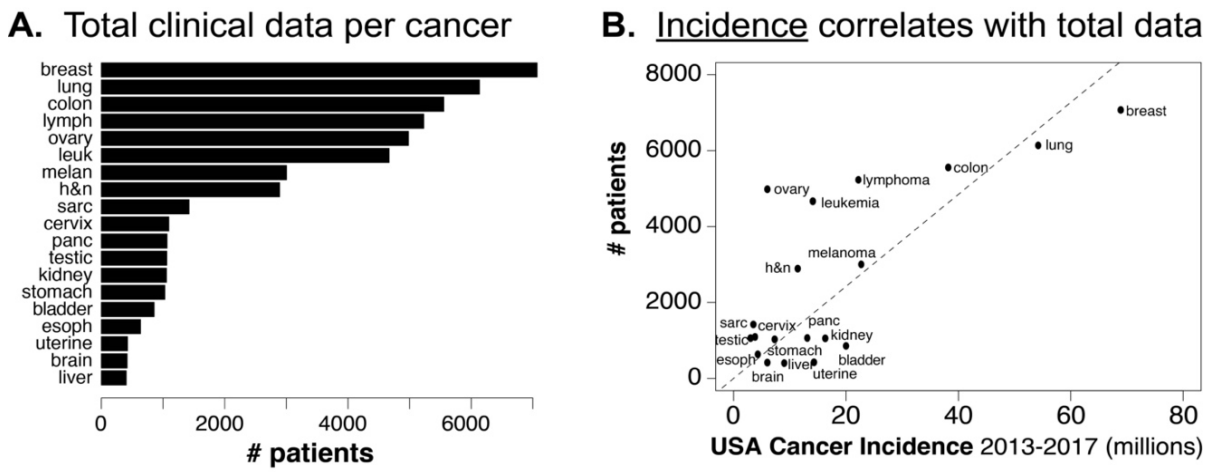


Figure 9. A. The total amount of curated patient data in the monotherapy database. **B.** US cancer incidence rates from 2013-2017 based off the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program (54). The total available patient data correlated with cancer incidence rates, which served as the driving factor when choosing cancers for this project.

robust to calculate the ORR of a monotherapy that has 10,000 patients across 100 studies than it is to calculate an ORR on a monotherapy with 700 patients and 7 studies. Based on the clinical data obtained from the monotherapy database curation, the top 8 cancers with the most patient data were: Breast, Non-Small Cell Lung, Colorectal, Lymphoma, Ovarian, Leukemia, Melanoma, and Head and Neck Cancer (**Fig. 9A**).

Incidence rate was also accounted for when choosing cancers to pursue. According to USA Cancer Incidence Rates from 2013-2017 (54), the top 8 cancers with the highest incidence correlated with the total clinical data (**Fig. 9B**). Since the data for the monotherapy database was curated from a textbook published in 1970, it was important to assess current cancer trends to ensure that there would be a suitable amount of current clinical data for analysis. Of the 8 listed cancers, only 5 were pursued for this research due to the type of cancer treatment data available.

The five cancers evaluated were Breast, Non-Small Cell Lung, Colorectal, Ovarian and Head and Neck Cancer. The three cancers that were unexamined included melanoma, lymphoma, and leukemia. Each cancer will be discussed individually.

One main reason melanoma was excluded was due to the types of antitumor agents that were used in treatment. Preliminary findings demonstrated that first-line treatment of metastatic melanoma largely included the use of immunotherapies, such as monoclonal antibodies. Some of the drugs used included Ipilimumab, Nivolumab, and Bevacizumab. Although there were studies that used cytotoxic chemotherapies, the number of studies using cytotoxic chemotherapies was limited, therefore, provided a weak dataset. Another limitation was due to the previously mentioned gaps of data in the monotherapy database. Although there were some combination chemotherapy studies available, there were missing single agent values in the monotherapy database. Therefore, a comparison between theoretical and clinical ORRs could not be made because there were missing monotherapy values available to calculate a theoretical response rate. However, this process was helpful in identifying specific gaps in the monotherapy database for cancer with a high incidence rate, and these gaps can be filled using literature searches in future work.

Blood cancers, such lymphoma, and leukemia, were not pursued for three reasons. One reason was due to the overall complexity of treatment regimens and dosing schedules, which posed challenges when comparing clinical studies. For example, upfront induction with specific therapy for Acute Myeloid Leukemia (AML) may utilize a 7+3d schedule, which is defined as 7 days of Drug A plus 3 days of Drug B. This regimen was only recommended for patients that were classified as “fit” due to the aggressive nature of the treatment. Patients who were classified as “unfit” received alternative chemotherapy options, that included monoclonal antibodies such as Gemtuzumab (55). This leads into the second reason for exclusion, which is that this project

aimed to look at the ORRs of only cytotoxic chemotherapy drugs and did not focus on targeted or immunotherapies. Preliminary findings also demonstrated that many of the clinical studies for ALM involved treating patients with a combination of cytotoxic drugs with other types of antitumor agents such as Gemtuzumab.

The third reason for excluding leukemia and lymphoma were due to the gaps in data in the monotherapy database and a lack of current cytotoxic combination data. Collectively, the monotherapy datasets for leukemia and lymphoma had adequate number of patients and studies. However, preliminary findings demonstrated that these therapies were not as common in current clinical practices. Due to a shifting paradigm and emerging popularity of monoclonal antibodies, the quantity of cytotoxic chemotherapy data was limited (55). Although there were some cytotoxic chemotherapy trials, these single agents were not included in the monotherapy database. This preliminary comparison identified additional gaps in the monotherapy database and provided future considerations to keep in mind when curating data on blood cancers.

Subsection 3: Combination Chemotherapy Database

The combination chemotherapy database was built using a similar encoding method to the monotherapy database (**Fig. 10**). Important information that was documented in the database included relevant clinical trial information such as drugs under investigation, size of patient cohort, and ORR values. Clinical studies were curated from the highly reputable hematology and oncology Wikipedia website HemOnc.org. This website, which was first created in 2011, aimed to fulfill the unmet need of a central repository of open access clinical studies (55,56). HemOnc.org represents the current clinical working knowledge for cancer treatment. Although HemOnc is a crowd source resource, all studies are extensively reviewed by an editorial board before being published on the website. Additionally, all studies are under continuous peer review to ensure validity and accuracy are maintained. Lastly, only verified practicing healthcare

professionals such as hematologists and oncologists, are allowed to submit studies for publishing on HemOnc.org. Currently, there are 5906 studies and 5652 regimen variants, but this number is continually increasing (55).

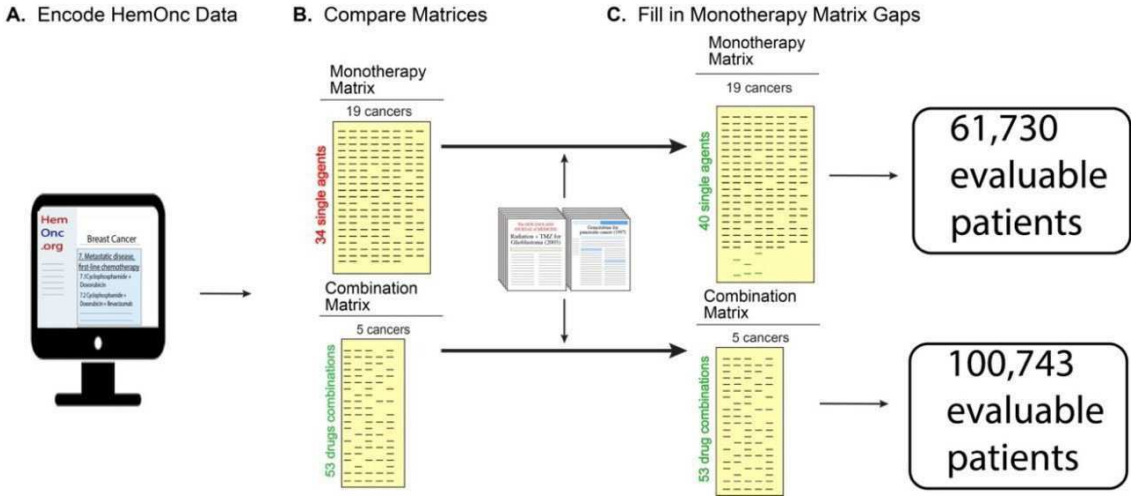


Figure 10. A. HemOnc.org was the primary resource used for curating clinical data. **B.** After an initial curation process for each matrix, comparison of drugs in both the monotherapy and combination databases were made to identify gaps. **C.** Several gaps in the monotherapy database were filled for analysis, increasing the total number of evaluable patients by an additional 12,728.

HemOnc is organized by 5 main categories: Solid Tumors, Malignant Hematology, Pediatrics, Transplant, and Classical Hematology. Each main category is further organized into site-specific subcategories, such as breast or colon oncology. Each subcategory includes all cancer phenotypes for which there is available data. For example, breast oncology includes ER-Positive, BRCA mutated, and Triple Negative Breast Cancer (55). Each cancer phenotype has its own specific Wikipedia page and is further categorized based on the treatment type. For example, breast cancer is organized based on neoadjuvant therapy, adjuvant therapy, metastatic disease first-line therapy, metastatic disease with subsequent lines, and so forth.

The scope of this research was to explore ORRs for combination cytotoxic chemotherapies, therefore, this project analyzed studies that used first-line treatment (Chemotherapy naïve patients) for advanced/metastatic cancer. Based on the previous discussion of cancer selection, the specific cancers that were evaluated are demonstrated in **Figure 11**.

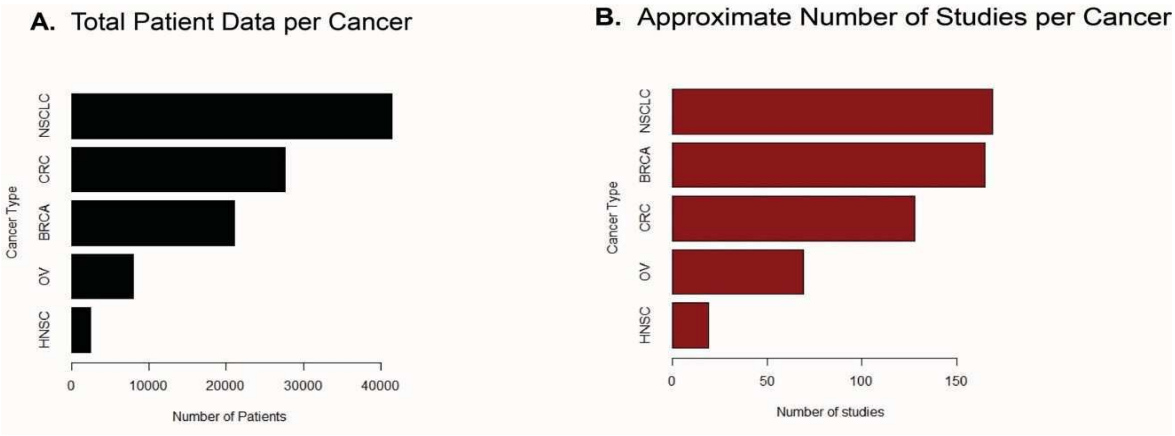


Figure 11. A. The total amount of curated patient data in the combination database. **B.** The total number of studies available on HemOnc.org for first-line treatment of advanced disease. This number at the time of this study, which may change due to studies being continuously added to HemOnc.org.

After data curation was complete, analysis was conducted on ORRs from 100,743 patients across 550 clinical studies. Studies were excluded from analysis if they included other noncytotoxic antitumor agents (ex. Monoclonal antibodies), did not report ORR values, or did not have ORR as the primary endpoint. It was also found that studies that did not include ORR as a primary endpoint did not always use standardized ORR criteria, such WHO criteria and RECIST. The data for these studies were included in the master database for each cancer, but the ORR values were excluded during the final analysis, which is discussed in Chapter 4: Results.

CHAPTER 4

ANALYSIS

Chapter Overview: This chapter will discuss the results for each cancer. The first subsection will define the three possible response relationships between monotherapies and combinations which are: Synergy, Independent Action, Antagonism. Each of the cancers pursued in this study will have a graph demonstrating the response relationship and a table that contains drug names, abbreviations, theoretical response rates, clinical response rates, and total number of evaluable patients for each drug combination. After subsection 1, the cancers will be discussed as follows: Subsection 2: Breast Cancer, Subsection 3: Non-small cell lung cancer, Subsection 4: Ovarian Cancer, Subsection 5: Colorectal cancer, and Subsection 6: Head and Neck Cancer. The results of this project demonstrated that the analyzable cancers expressed considerable disagreement with independent drug action by either producing underpredicted or overpredicted response rates.

Subsection 1: Defining Graphical Relationships

As previously mentioned in Chapter 3: Database Building, the primary endpoint data that was collected and analyzed for this study were Objective Response Rates (ORRs). ORR values were obtained for each clinical trial that met the scope of this study. To reiterate, the studies that met the criteria were performed as a first-line chemotherapy for metastatic/advanced disease. This meant that patients were chemotherapy naive prior to enrollment in a clinical trial. In addition to ORR values, the number of patients evaluated in each study was also documented. By doing so, weighted average calculations could be performed to generate an overall response rate value for the combination chemotherapy. This value is defined as the clinical response rate for

the purposes of this study. Weighted averages were also used to calculate a single overall response rate value for each monotherapy drug. As previously mentioned, this value was used in the Fractional Kill Hypothesis equation to generate a theoretical response rate for a drug combination. Theoretical response rates (independent variable) were plotted against the corresponding clinical response rate (dependent variable) to examine the relationship between the expected response rate versus the actual clinical outcome reality for a given combination. The possible graphical outcomes are illustrated by **Figure 12**. In brief, if a response rate value is plotted along the black diagonal line, then this drug-cancer paring demonstrate agreement with the Fractional Kill Hypothesis and independent action. If a response rate value falls above or below the diagonal line, then the drug-cancer pairing does not exhibit agreement. The theoretical and clinical response rates were determined for the cancers outlined in Chapter 3 and the results of each cancer will be discussed individually. The first cancer that will be discussed is breast cancer.

All the data analysis and associated figures in this thesis were conducted using R statistical software, which is free to download at https://www.r-project.org. Theoretical response rates were calculated using the Fractional Kill Hypothesis Equation (**Fig. 5**) and were plotted on the X-axis. Clinical response rates were calculated using the weighted averages of monotherapy and combination chemotherapy response rates, and these values were plotted on the Y-axis. Scatter plots were generated in R using the function "ggplot", which found in the ggplot2 package. This technique was used to generate a plot for each cancer to conduct the analyses.

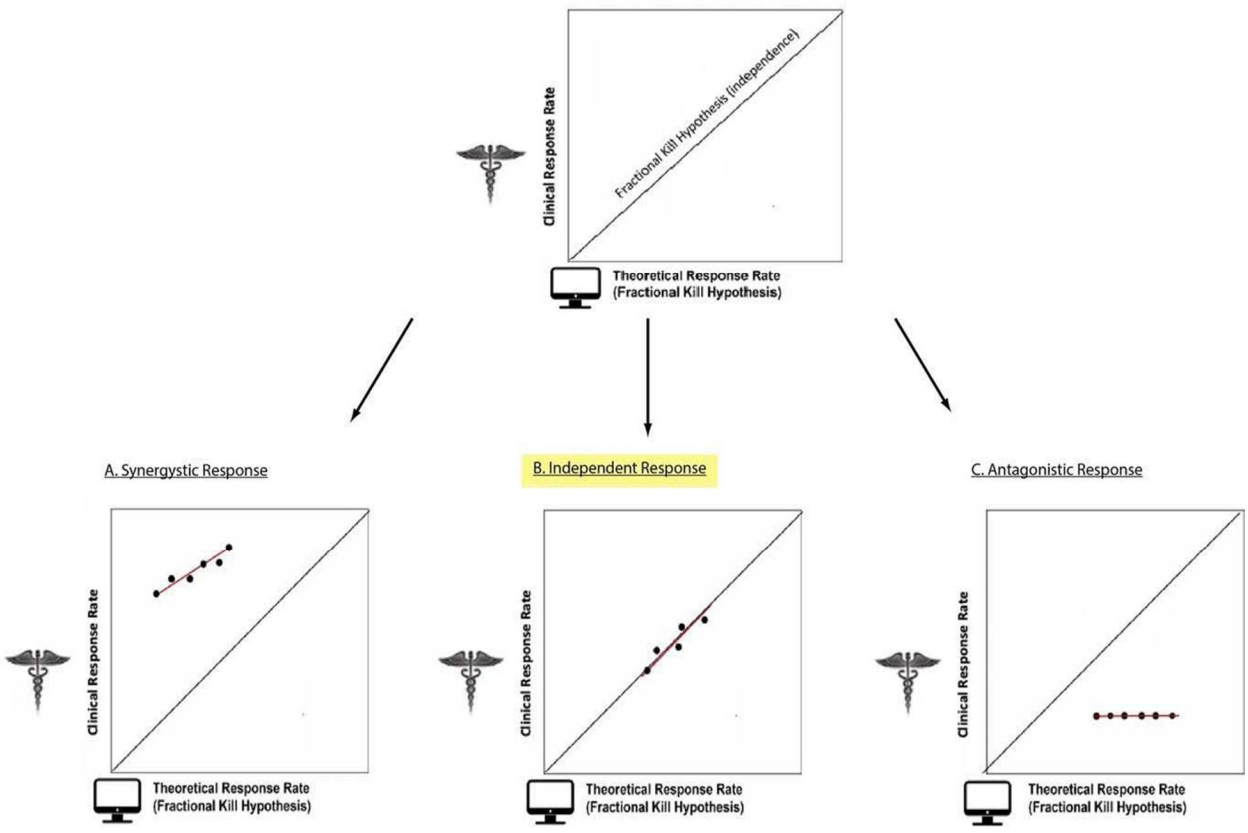


Figure 12. This plot (top) demonstrates the setup for graphical analysis of each study. The black line running diagonally through the plot represents the clinical assumption of independent drug action as explained by the Fractional Kill Hypothesis. There are one of three possible relationships can occur when comparing theoretical and clinical Response Rates (RR): **A.** Synergistic ($RR_{Clinical} > RR_{Theoretical}$). **B.** Independent ($RR_{Clinical} = RR_{Theoretical}$). **C.** Antagonistic ($RR_{Clinical} < RR_{Theoretical}$). The Independent relationship (**B**) illustrates the underlying clinical assumption of the Fractional Kill Hypothesis, which is that a combination of drugs exhibits independent action. Combinations that show agreement will be plotted along the black diagonal line.

Subsection 2: Breast Cancer

The type of breast cancer explored in this project was metastatic disease and did not include any specific subtypes of breast cancer, such as Human Epidermal Growth Factor Receptor 2 (HER2) positive or Triple Negative Breast Cancer, which is breast cancer that exhibits negative immunohistochemical testing results for the estrogen, progesterone, and HER2 receptors. The justification for this decision was to obtain breast cancer data that was comparable to the breast cancer monotherapy data extracted from *Single Agents in Cancer Chemotherapy*. The breast cancer dataset contained ORR values for 18 unique drug pair combinations and 4 monotherapy drugs. After filtering through 161 clinical trials, the total number of patients analyzed in this dataset was 21,295. The classification of drugs used in this dataset included alkylating agents anthracyclines, taxanes, antimetabolites, and vinca alkaloids.

Table 1. This is a table of the analyzed drugs from the breast cancer combination chemotherapy database. Information included is drug names, abbreviations (abbrev.), clinical response rates, theoretical response rates, and the total number of evaluable patients for the drug combination. See **Fig.12** for the graphical relationship between monotherapy and combination chemotherapy response rates. The data is sorted by **THEORETICAL**, descending from highest to lowest expected response rate.

DRUG NAMES	ABBREV.	CLINICAL	THEORETICAL	TOTAL PATIENTS
Cyclophosphamide_Doxorubicin_Docetaxel	CAT	0.35	0.83	146
Cyclophosphamide_Doxoubicin_Fluorouracil_ Methotrexate	CAFM	0.50	0.80	105
Cyclophosphamide_Methotrexate_Fluorouracil _Vinblastine	CMFVb	0.62	0.74	50
Doxorubicin_Docetaxel	AT	0.54	0.74	651
Docetaxel_Epirubicin	TE	0.52	0.72	326
Docetaxel_Gemcitabine	TG	0.41	0.71	699
Cyclophosphamide_Doxorubicin_Fluorouracil	CAF	0.48	0.69	1982
Cyclophosphamide_Methotrexate_Fluorouracil	CMF	0.38	0.68	1263
Gemcitabine_Epirubicin_Paclitaxel	GEP	0.62	0.67	114
Cyclophosphamide_Epirubicin_Fluorouracil	CEF	0.48	0.66	2387
Cyclophosphamide_Doxorubicin	CA	0.45	0.59	1049
Docetaxel	T	0.51	0.59	1251
Doxorubicin_Paclitaxel	AP	0.37	0.58	717
Cyclophosphamide_Epirubicin	CE	0.51	0.55	534
Epirubicin_Paclitaxel	EP	0.53	0.54	1210
Doxorubicin_Fluorouracil	AF	0.43	0.53	105
Paclitaxel_Gemcitabine	PG	0.41	0.52	508
Doxorubicin_Vincristine	AVc	0.55	0.50	218
Epirubicin_Vindesine	EVd	0.46	0.44	61
Doxorubicin	A	0.35	0.37	704
Paclitaxel	P	0.31	0.33	5850
Epirubicin	E	0.40	0.31	1365

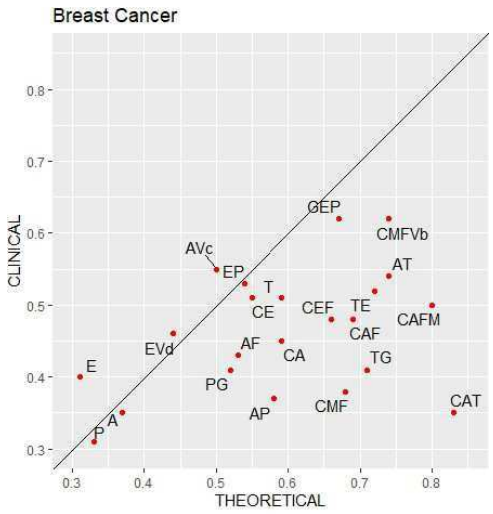


Figure 13. Graph comparing theoretical (x) and clinical (y) response rates for NSCLC. Black solid line represents independent action. Response rates values along the axes are listed as fractions rather than percentages.

The results of plotting theoretical response rates versus clinical response rates are shown in F**igure 13**. The diagonal black line is a visual representation of independent drug action, which is the underlying assumption of the Fractional Kill Hypothesis and Dr. Sorger's findings. If a combination of drugs were exhibiting independent action, the X values (theoretical response rates) would agree with the Y values (clinical response rates) meaning that these values would be plotted along or remarkably close to the diagonal line. Referring to the earlier example using the AT combination in Chapter 2, the clinical response rate was 54%, but based on the Fractional Kill Hypothesis equation, the theoretical response rate was 20% higher at 74%. However, what **Figure 13** demonstrates, is that the AT combination, along with most of the drug combinations in this dataset, exhibits an observable amount of disagreement. Out of 19 combination datapoints, 3 of these drug pairings showed possible agreement with independent action. Intriguingly, two of these three drugs involved the combination of anthracyclines and vinca alkaloids. Speculative reasons explaining sources of non-independence and resistance mechanisms will be addressed in greater detail in Chapter 5: Discussion

The monotherapy values were included in the graphical analysis and served as internal controls. Monotherapy ORR data was extracted from HemOnc using the previously described

processes to generate an overall clinical response rate value. The HemOnc monotherapy values, which are from more recent clinical trials, were compared to the ORR datasets from the monotherapy database to confirm the validity of the NCI 1970 monotherapy ORR values. As evident from the graph (**Fig. 13)**, the monotherapy data from the NCI 1970 textbook does agree closely with the monotherapy data from HemOnc.org, especially in the cases of Doxorubicin and Paclitaxel. It was important to include this internal control to exhibit the validity and credibility of the theoretical response rate values, since these values were generated using the monotherapy data extracted from the NCI 1970 textbook.

Subsection 3: Non-Small Cell Lung Cancer (NSCLC)

The next cancer that will be discussed is NSCLC, specifically looking at first-line treatment for metastatic/advanced disease. Similarly with breast cancer, there was no specific histological or mutagenic subtype that was specifically pursued for the interest of maintaining consistency with the monotherapy database. At the time of the final analysis, there were 24 unique combinations of drugs and 7 monotherapy drugs included in the graphical analysis. 79% (19/24) of the drug combinations in the NSCLC dataset involved the pairing of a platinum drug, such as cisplatin and carboplatin, with vinca alkaloids, antimetabolites, anthracyclines, and taxanes. Only 5 of the 24 unique drug pairings did not involve the use of a platinum-based drug. After filtering through 169 clinical trials, the total number of patients analyzed in this dataset was 41,486.

As previously mentioned, the monotherapy drugs were included as an internal control to ensure consistency between the NCI 1970 monotherapy dataset and more current clinical trials.

Having this internal control was beneficial, as it allowed for validation of the values for the two most used single agent drugs, cisplatin and carboplatin. Validation of these monotherapy values required datamining efforts outside of HemOnc due to limiting or missing monotherapy data available for each of these drugs on the website. The reputable search engine Google Scholar was used to find high quality and credible studies by using phrases such as "First-line treatment of Non-Small Cell Lung Cancer with the monotherapy/single-agent Carboplatin/Cisplatin". After finding additional studies for each drug, the ORR values were validated using the pre-existing monotherapy data, and final clinical response rates were calculated.

A similar curation process was also followed to generate an ORR value for one of the most used vinca alkaloids found in this project, which was vinorelbine. Vinorelbine monotherapy data was important for the analysis of 4 of the 24 unique combinations of drugs for NSCLC. As mentioned in Chapter 3, there have been several gaps in the monotherapy database that have been identified and filled during the process of combination chemotherapy database building and analysis. At the time of this project, vinorelbine monotherapy data did not exist in our monotherapy database. Vinorelbine monotherapy data was curated from HemOnc.org following the same methodologies and was added to the monotherapy database.

Table 2. This is a table of the analyzed drugs from the non-small cell lung cancer combination chemotherapy database. Information included is drug names, abbreviations (abbrev.), clinical response rates, theoretical response rates, and the total number of evaluable patients for the drug combination. See **Fig.14** for the graphical relationship between monotherapy and combination chemotherapy response rates. The data is sorted by **THEORETICAL** (descending).

DRUG NAMES	ABBREV.	CLINICAL	THEORETICAL	TOTAL PATIENTS
Cyclophosphamide_Doxorubicin_Lomustine_ Methotrexate	CALM	0.32	0.70	80
Cisplatin_Ifosfamide_Mitomycin	CPIMC	0.40	0.53	1309
Cisplatin_Gemcitabine_Ifosfamide	CPGI	0.40	0.52	826
Cisplatin_Cyclophosphamide_Doxorubicin	CPCA	0.14	0.52	380
Cisplatin_Ifosfamide_Vindesine	CPIVD	0.47	0.50	100
Docetaxel_Gemcitabine	TG	0.31	0.49	944
Carboplatin_Cisplatin_Ifosfamide	CBCPI	0.29	0.47	332
Gemcitabine_Ifosfamide	GI	0.26	0.45	323
Cisplatin_Docetaxel	CPT	0.32	0.44	2238
Carboplatin_Docexatel	CBT	0.28	0.44	898
Cisplatin_Gemcitabine_Vinorelbine	CPGVR	0.40	0.43	463
Cisplatin_Mitomycin_Vindesine	CPMCVD	0.33	0.43	476
Carboplatin_Gemcitabine_Paclitaxel	CBGP	0.34	0.40	492
Cisplatin_Mitomycin_Vinblastine	CPMCVB	0.30	0.39	671
Docetaxel	T	0.16	0.36	909
Gemcitabine_Vinorelbine	GVR	0.24	0.35	1320
Gemcitabine_Paclitaxel	GP	0.32	0.32	1359
Cisplatin_Gemcitabine	CPG	0.31	0.31	6541
Carboplatin_Gemcitabine	CBG	0.28	0.30	1728
Cisplatin_Vinorelbine	CPVR	0.30	0.29	3557
Cisplatin_Vindesine	CPVD	0.26	0.27	742
Cisplatin_Paclitaxel	CPP	0.28	0.25	1557
Carboplatin_Paclitaxel	CBP	0.27	0.25	9452
Cisplatin_Vinblastine	CPVB	0.18	0.23	409
Cisplatin_Etoposide	CPE	0.20	0.21	836
Gemcitabine	G	0.18	0.20	772
Vinorelbine	VR	0.18	0.18	1115
Vindesine	VD	0.10	0.16	143
Paclitaxel	P	0.16	0.14	414
Cisplatin	CP	0.13	0.13	1100

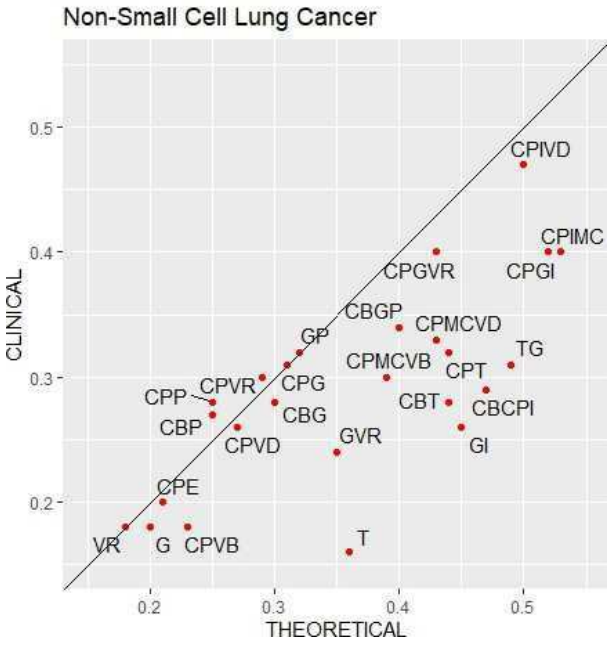


Figure 14. Graph comparing theoretical (x) and clinical (y) response rates for NSCLC. Black solid line represents independent action. Response rates values along the axes are listed as fractions rather than percentages.

The NSCLC dataset, as were all the datasets in this research project, was plotted in a similar way to the breast cancer dataset (**Fig. 14**). Upon initially looking at the graph, the NSCLC dataset would appear to be in more agreement with independent action than the BRCA dataset. However, it is important to ignore the monotherapy values, because after the previously mentioned curation processes, it is expected that these values would fall along the solid black line (which indicates independent action). The graphic demonstrates that only 5 of the 24 unique combinations (21%) fall along or above the line of independent action. While this value is lower than the BRCA dataset (30%), it was important to note that the theoretical response rates for NSCLC were comparatively lower than the theoretical response rates for BRCA dataset.

To expand on this observation, 17 out of 18 (94%) of the drug combinations in the BRCA dataset were expected to have a theoretical response rate of 50% or greater. The one only combination with a theoretical response rate lower than 50% was Epirubicin and Vindesine. In contrast, only 4 out of 24 (16%) of the drug combinations within the NSCLC dataset had a theoretical response rate above 50%. Only one of these combinations, Cyclophosphamide, Doxorubicin, Lomustine, Methotrexate (CDLM), had a theoretical response rate above 53%.

Comparatively, the final clinical response rates for NSCLC were low, with only one combination achieving a clinical response rate of greater than 40%. In addition to testing the current working Fractional Kill Hypothesis, this dataset also provided validation for the current clinical limitations of treating NSCLC with cytotoxic chemotherapies. From this dataset, it can be inferred that NSCLC is less responsive to cytotoxic chemotherapy, especially at least when compared to other cancers in this project.

Subsection 4: Ovarian Cancer

The next cancer that will be discussed is ovarian cancer. As with the previous two cancers (BRCA and NSCLC), there was no specific histological or mutagenic subtype that was specifically pursued for the interest of maintaining consistency with the monotherapy database. After filtering through 69 studies, the total number of patients analyzed in this dataset was 8,036. At the time of the final analysis, there were 14 unique combinations of drugs and 4 monotherapy drugs included in the graphical analysis, for a total of 18 drugs in the matrix. 79% (11/14) of the drug combinations in the Ovarian dataset involved the pairing of the platinum drugs, cisplatin and carboplatin, with antimetabolites, anthracyclines, and taxanes. Only 3 of the 14 unique drug pairings did not involve the use of a platinum-based drug.

Interestingly, one clinical trial investigated a combination of carboplatin and cisplatin. However, this combination produced underwhelming results, with only 38% of the 115 patients producing an objective response. Retrospectively, this response can be seen as rather unsurprising. Per the classical algorithm of drug design mentioned in Chapter 2, the clinical assumption is that an effective combination of drugs involves two or more drugs of different mechanisms of action. Therefore, it would be inferred that using two drugs of the same mechanism of action, would not produce any greater results. When looking at the rest of the dataset, Ovarian has the highest expected theoretical response rate out of all cancers explored in

this project. 15/18 (83%) of the drugs in the dataset had a theoretical response rate of 63% or greater. It is important to note that one of these values involved the combination of cisplatin and carboplatin, but this value does not follow the fractional kill hypothesis's underlying assumption of independent action. After finishing the curation process for the previous two cancers, monotherapy drugs continued to be included as an internal control. This internal control was beneficial when comparing the values of two drugs: melphalan and paclitaxel. Luckily, there was sufficient monotherapy data available for both drugs on HemOnc.org. The monotherapy values curated from HemOnc were used to validate the single values within the monotherapy database.

Table 3. This is a table of the analyzed drugs from the ovarian cancer combination chemotherapy database. Information included is drug names, abbreviations (abbrev.), clinical response rates, theoretical response rates, and the total number of evaluable patients for the drug combination. See **Fig.15** for the graphical relationship between monotherapy and combination chemotherapy response rates. The data is sorted by **THEORETICAL** (descending).

DRUG NAMES	ABBREV.	CLINICAL	THEORETICAL	TOTAL PATIENTS
Cisplatin_Cyclophosphamide_Doxorubicin	CPCA	0.56	0.86	32
Cisplatin_Doxorubicin_Paclitaxel	CPAP	0.64	0.86	164
Carboplatin_Cisplatin	CBCP	0.38	0.84	114
Carboplatin_Epirubicin_Paclitaxel	CBEP	0.74	0.82	352
Carboplatin_Gemcitabine_Paclitaxel	CBGP	0.86	0.81	182
Cisplatin_Cyclophosphamide	CPC	0.53	0.79	872
Cisplatin_Paclitaxel	CPP	0.66	0.79	767
Carboplatin_Cyclophosphamide	CBC	0.60	0.76	135
Carboplatin_Paclitaxel	CBP	0.58	0.75	3256
Carboplatin_Docetaxel	CBT	0.59	0.70	300
Fluorouracil_Melphalan_Methotrexate	FMELM	0.40	0.69	57
Carboplatin_Gemcitabine	CBG	0.68	0.66	139
Cisplatin	CP	0.63	0.63	174
Cyclophosphamide_Doxorubicin	CA	0.58	0.63	299
Fluorouracil_Melphalan	FMEL	0.30	0.63	77
Carboplatin	CB	0.52	0.57	702
Paclitaxel	P	0.43	0.43	131
Melphalan	MEL	0.38	0.38	283

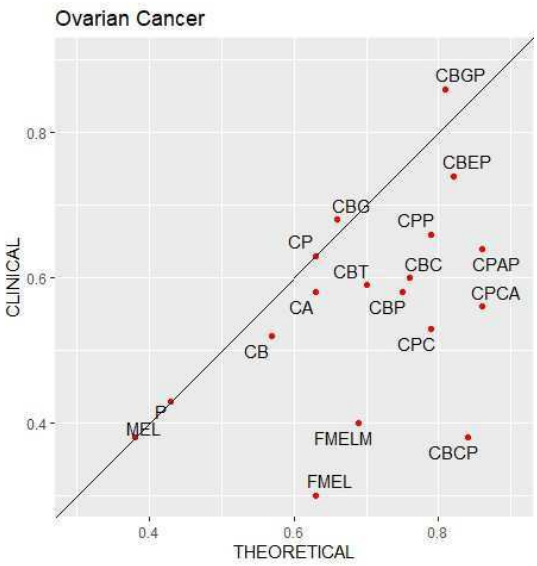


Figure 15. Graph comparing theoretical (x) and clinical (y) response rates for Ovarian cancer. Black solid line represents independent action**.** Response rates values along the axes are listed as fractions rather than percentages.

Based on this dataset, Ovarian cancer had the highest average clinical response rates to cytotoxic chemotherapy treatment out of all analyzed cancers (**Fig. 15**). Also, it is worth noting that both NSCLC and ovarian, which were mostly treated with platinum-based combination chemotherapy regimens, had significantly opposing theoretical and clinicals response rates. As a comparison, only 4 platinum drug combinations of the NSCLC had clinical response rates of greater than or equal to 40%. In contrast, all platinum drug combinations within the ovarian cancer dataset had clinical response rates of greater than 50% (with exception to the cisplatin and carboplatin combination that was previously discussed).

Although the ORRs of ovarian cancer to cytotoxic chemotherapy were higher, the graphical representation demonstrated that there is still disagreement with independent action. The only datapoint that produced results that would indicate independent action was a 3-drug combination of carboplatin, gemcitabine, and paclitaxel. The higher ORRs for ovarian cancer demonstrate that more patients are more likely to respond to first-line treatment, which other studies have also shown (57). However, the clinical response rates still produce underpredicted results, thus warranting further investigation.

Subsection 5: Colorectal Cancer

The final cancer that will be discussed is metastatic/advanced colorectal cancer treated with first-line chemotherapy. As with all the previous cancers, no specific histological subtype of cancer was pursued, and the disease had to be metastatic or advanced. After filtering through 128

clinical trials, the total number of patients analyzed in this dataset was 27787. At the time of analysis, there were 16 unique drug combinations, 4 single-agent drugs, and 1 formulation of fluorouracil and leucovorin (FULV). For the purposes of this study, FULV was considered a monotherapy drug. Leucovorin, also known as folinic acid, acts as a chemotherapy modulating agent. Leucovorin is not considered a chemotherapy drug due to its role in either increasing the effects of antitumor effects of a drug or decreasing toxicity. Additionally, there were no studies using only leucovorin curated from the NCI 1970 textbook, HemOnc, or in a Google Scholar web search. As such, leucovorin alone is not included in this dataset.

Another reason why FULV was not included in the fluorouracil monotherapy data was largely in part because many studies compared the efficacy of fluorouracil alone versus FULV. Subsequently, each of these two formulations (FULV or fluorouracil alone) generated separate response rates. Therefore, these values were recorded in the combination chemotherapy database separately. Since FULV and fluorouracil may have different ORRs, the drugs would generate different theoretical response rates as calculated using the mathematical process described in Chapter 2. Additionally, multiple combinations in the database either use only fluorouracil in combination with other cytotoxic agents (ex. fluorouracil and irinotecan) or use FULV with other cytotoxic agents (ex. fluorouracil, leucovorin and irinotecan). Having different ORR monotherapy values for fluorouracil and FULV would impact the theoretical response rate values calculated using these drugs.

Table 4. This is a table of the analyzed drugs from the colorectal cancer combination chemotherapy database. Information included is drug names, abbreviations (abbrev.), clinical response rates, theoretical response rates, and the total number of evaluable patients for the drug combination. See **Fig.16** for the graphical relationship between monotherapy and combination chemotherapy response rates. The data is sorted by **THEORETICAL** (descending).

DRUG NAMES	ABBREV.	CLINICAL	THEORETICAL	TOTAL PATIENTS
Fluorouracil_Leucovorin_Irinotecan_Oxaliplatin	FOLFOXIRI	0.54	0.49	259
Fluorouracil_Mitomycin_Semustine	FMCS	0.06	0.41	62
Capecitabine_Irinotecan	CI	0.41	0.38	554
Capecitabine_Oxaliplatin	COX	0.41	0.38	934
Dacarbazine_Fluorouracil_Semustine	DFS	0.15	0.38	101
Fluorouracil_Leucovorin_Lomustine	FOLFL	0.22	0.38	145
Fluorouracil_Irinotecan	FI	0.49	0.37	515
Fluorouracil_Oxaliplatin	FOX	0.53	0.37	515
Fluorouracil_Leucovorin_Irinotecan	FOLFIRI	0.40	0.36	4802
Fluorouracil_Leucovorin_Oxaliplatin	FOLFOX	0.48	0.36	4896
Irinotecan_Oxaliplatin	IOX	0.38	0.36	460
Fluorouracil_Mitomycin	FMC	0.34	0.34	279
Fluorouracil_Methotrexate	FM	0.05	0.33	21
Fluorouracil_Leucovorin_Methotrexate	FOLFM	0.24	0.31	78
Fluorouracil_Hydroxyurea	FH	0.18	0.30	85
Fluorouracil_Semustine	FS	0.23	0.30	315
Capecitabine	C	0.22	0.22	1450
Fluorouracil	F	0.22	0.22	3291
Fluorouracil_Leucovorin	FOLF	0.20	0.20	8032
Oxaliplatin	OX	0.20	0.20	188
Irinotecan	I	0.18	0.18	226

Although the curation of the combination database was relatively straightforward, there were several gaps in the monotherapy database that required additional literature searches. Specifically, monotherapy ORR values were generated for 4 of the 4 monotherapy agents: FULV, capecitabine, irinotecan, oxaliplatin, by using HemOnc.org and through conducting literature searches. This additional curation process was necessary for conducting the analysis because 55% (10/16) of the combinations in this dataset used one of the four previously mentioned drugs. The process for obtaining data for FULV and capecitabine was straightforward, as there were an extensive number of studies included in HemOnc. The final clinical response rate for both drugs were calculated using these curated papers and the values were incorporated into the monotherapy dataset for further analysis.

In contrast with FULV and capecitabine, the curation process for irinotecan and oxaliplatin involved literature searches since these drugs were all approved for treatment of colorectal cancer after the publication of the *Single Agents in Cancer Chemotherapy.* This was due to only one monotherapy study for irinotecan and zero oxaliplatin monotherapy studies being published on HemOnc.org. Therefore, an extensive literature search was conducted for both irinotecan and oxaliplatin. The process was similar to the process when searching for papers on treating NSCLC with carboplatin/cisplatin. In Google Scholar, phrases such as, or similar to, the following were used: “First-line treatment of Colorectal cancer with the monotherapy/single-agent Irinotecan/Oxaliplatin”. After finding several studies and metareviews for both agents, monotherapy ORR values were generated using the weighted average method as previously described. These values were incorporated into the monotherapy database and used to generate “Theoretical Response Rates” for the remainder of the analysis.

To conclude this discussion of the monotherapy drugs, a final remark will be made about the fluorouracil monotherapy data. Surprisingly, the final clinical response rate value for fluorouracil in the monotherapy database (21.5%) was in strong agreement with the monotherapy value generated by data curation process of the combination database (22.3%). Therefore, this finding instills confidence in the validity of the fluorouracil monotherapy values used throughout the analysis of this dataset.

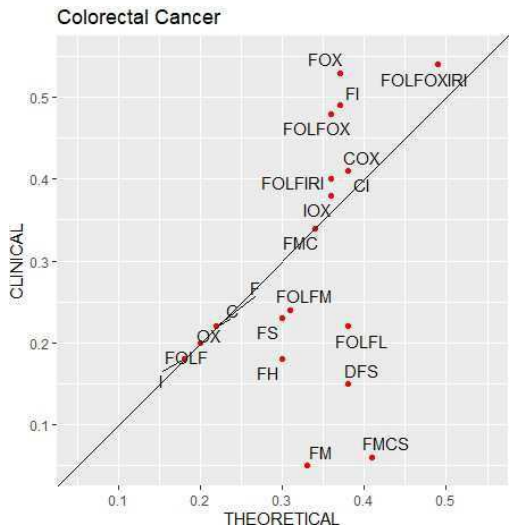


Figure 16. Graph comparing theoretical (x) and clinical (y) response rates for CRC. Black solid line represents independent action. Response rates values along the axes are listed as fractions rather than percentages.

Based on this dataset, the overall findings differed from the three previously discussed cancers (**Fig. 16**). Breast, NCSCL, and ovarian cancer all showed considerable disagreement with independent action by falling below the diagonal line. Colorectal cancer exhibits disagreement with independent action by falling above and below the diagonal line. 44% (7/16) of the clinical response rates for colorectal cancer were greater than the theoretical response rates. Interestingly, all 8 of the drugs that performed better than predicted involved a combination of irinotecan, oxaliplatin, or both, with FULV or fluorouracil. Contrarily, the combinations that generated clinical response rates that were lower than the theoretical response rates did not use irinotecan or oxaliplatin. It is intriguing to note the differences in response rates when treating colorectal cancer with irinotecan and oxaliplatin versus any of the other drugs. Irinotecan is the only drug that is a topoisomerase I inhibitor that was used in this combination dataset. Likewise, oxaliplatin was the

only platinum-based therapy used to treat colorectal cancer in this dataset. Therefore, it is worth exploring the relationship between irinotecan, oxaliplatin and colorectal cancer biology to better understand these response rates.

Subchapter 6: Head and Neck Cancer

It is worth mentioning that curation and analysis processes were attempted with head and neck cancer. Unfortunately, there was an insufficient number of studies than originally anticipated. At the time of this curation, there were only 23 evaluable studies on HemOnc. From this, data was extracted on 7 unique drug combinations and three monotherapy drugs used on a total of 2,415 patients. This caused an issue for conducting analysis, since these ORRs did not have a sufficient number of studies to support these values.

For example, there was only one study available that used carboplatin and docetaxel to treat head and neck cancer, in which the clinical response rate was 17%. Since this was the only study that used this combination, there were no other studies available to generate a weighted clinical response rate. Therefore, there was only one study that supported this low clinical response rate. This was the case for 6 additional sets of drugs in the dataset, which accounted for 70% of the dataset. Because of insufficient data, any claims made in the analysis were unable to be supported.

There was another limitation encountered with the head and neck cancer dataset, which was the difference in single-agent ORRs between the monotherapy and combination datasets for cisplatin and methotrexate. For the combination datasets, these drugs had the most amount of clinical data to support the clinical response rates. However, these values greatly differed from the monotherapy database by over double where the clinical response rate for Cisplatin was 30% versus 14% and for Methotrexate: 42% versus 16% for combination database versus monotherapy database sources, respectively. Therefore, it would be worthwhile to normalize the

ORR values generated in the monotherapy database with newer monotherapy data. The single agent values obtained in the combination dataset could be combined with the single agent values in the monotherapy database through weighted averages to generate a more accurate single agent value for both cisplatin and methotrexate. Despite the apparent limitations, it does allow for opportunities for further investigation in future work as preliminary response data has been recorded. A substantial number of head and neck cancer studies would need to be curated via literature searches through Google Scholar or other search engines using methods similar to those used in the curation process of previously discussed cancers. However, this is a time-consuming process that was not able to be completely explored due to time constraints of this program's deadlines.

To summarize the findings of this analysis, three of the four fully analyzed cancers, breast, NSCLC, and ovarian cancer, demonstrated disagreement with the Fractional Kill Hypothesis and independent drug action by producing underpredicted results. Although colorectal cancer produced better than expected results, this still demonstrates disagreement with independent action and Fractional Kill Hypothesis. Additionally, it demonstrates disagreement in Dr. Sorger's findings that combination chemotherapy drugs provide clinical benefits in the absence of synergistic drug action.

CHAPTER 5

DISCUSSION

Chapter 5: The first subsection aims to emphasize the relevance of this work as it pertains current cancer incidences. The second subsection will discuss how current clinical diagnostic techniques and overlapping drug resistance can be used to explain the results of this study. The third subsection will discuss how the scope of this work relates to ongoing and future work within the Douglass lab.

Subsection 1: Current Cancer Trends

Technological advancements over the past one hundred years have allowed for drastic improvement in cancer knowledge and creating more effective treatments (58). Identifying the hallmarks of cancer has enabled the development of newer and effective drugs. Some of these hallmarks describe the molecular mechanisms and pathophysiology of tumorigenesis, proliferation, and metastasis. Some of the ways in which tumor cells can accomplish these tasks is through acquired resistance to antigrowth factors and the capability to avoid apoptosis. This allows for the tumor cell to proliferate continually and uncontrollably (59). Advancements in cancer knowledge has contributed to the decreasing trend of cancer deaths over the past several decades. Per the CDC, in the past twenty years, the rate of cancer deaths in the US has dropped by 27% (60). Reasons for this decrease in cancer deaths can be attributed to the development of effective cancer screening tools, newer cancer treatments, and increased public awareness of cancer risk factors such as tobacco use, obesity, and predisposed genetic factors (61).

Despite efforts in creating better treatments, however, cancer is still the second leading cause of death in the United States, behind heart disease (62). Three of the five cancers in this

research project (breast, non-small cell lung, and colorectal cancer) were in the top 4 leading causes of cancer deaths in the United States during 2020 (60). Pancreatic cancer, which was the third leading cause of deaths in 2020 and is projected to be the second cause of cancer deaths, was beyond the scope of this project (63). However, lung cancer, was the number one leading cause of all cancer death, accounting for approximately 23% of all cancer deaths. Breast and colorectal cancer collectively accounted for 16% of cancer deaths. Thus, three of the five cancers explored in this research are responsible for almost 40% of all cancer deaths in the United States (62). On a global scale, WHO reported that respiratory cancers (ex. bronchial, tracheal and lung) were the 6th leading cause of death in 2020 (64). Although trends in cancer deaths are decreasing, incidence rates demonstrate that cancer still poses a major threat to humans from health and economic standpoints. Reasons for such poor clinical outcomes can be attributed to drug resistance, which may have sources of non-independent resistance. The purpose of my work beyond exploring and testing the Fractional Kill Hypothesis, was to guide ongoing experimental work within the Douglass lab specifically investigating the sources of non-independent drug resistance.

Subsection 2: Drug Resistance

Poor tumor response to chemotherapy drugs can be attributed to molecular mechanisms involved in cellular regulation, such as ATP- dependent drug efflux pumps (65). One well known multidrug resistant gene, ATP Binding Cassette Subfamily B Member 1 (ABCB1), which encodes the Multidrug Resistance Protein 1 (MDR1) known as P-glycoprotein (Pgp), has demonstrated resistance to a broad range of chemotherapy drugs. Pgp is part of larger class of ABC transporters, which are integral membrane proteins that can translocate substrates across cell membranes via ATP hydrolysis. ABC transporters exist ubiquitously and are involved in many physiological processes, such as regulating intracellular levels of substrates such as small

molecules (66,70). Although some ABC transporters have very specific substrate targets, there are several transporters, such as Pgp, that have a wide range of substrates that they can transport out of the cell. As a result, MDR1 can pump several common types of antitumor drugs out of intracellular space, such as anthracyclines, vinca alkaloids, and taxanes, which ultimately cause multidrug resistance (66).

Several studies have demonstrated that levels of Pgp intrinsically higher in certain cancers due to inherent anatomical differences between tissue types (65,66,69). For example, Pgp has a high expression in kidney tubular epithelial cells due to the physiological involvement in filtration of blood. Three of the four cancers explored in this project, breast, non-small cell lung, and colon cancers, are considered to have intrinsically higher expression levels of MDR1 (66). The expression of MDR1 in ovarian cancer, which was fourth cancer explored in this project, is present in less than 10% of ovarian cancers. However, evidence shows that ovarian tumors can develop high levels of MDR1 expression after initial exposure to chemotherapy or may be the cause of chemoresistance to certain drugs such as paclitaxel (66, 67). Additionally, MDR1 can be potentially used as a predictive biomarker for ovarian cancer response to treatment for those with overexpression. However, additional characterization of Pgp involvement in multidrug resistance is needed to design more effective combination.

Subsection 3: Ongoing and Future Work in the Douglass Lab

When experimentally designing combination chemotherapies, Loewe additivity and Bliss Independence are two methods used to describe the expected dose-response relationship for combination chemotherapies (50) (**Fig. 17**).

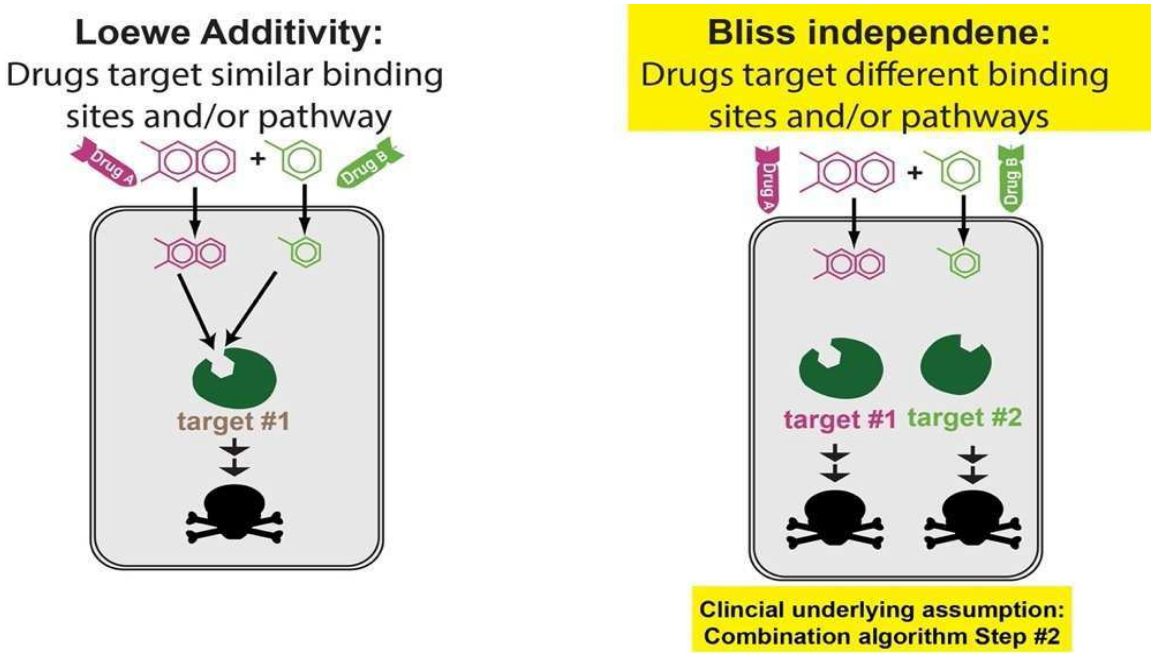


Figure 17. Left: Loewe additivity is described as two drugs that have similar mechanisms of action for cell killing. **Right**: Bliss independence is described two drugs that have dissimilar mechanisms of action, for cell killing. Bliss independence describes how clinical underlying assumption of independent drug action acts on a molecular level.

Loewe Additivity assumes that the two inhibitors involved in the combination have comparable mechanisms of action, which can act either noncompetitively or competitively towards the drug target. In contrast, Bliss Independence explains that combination chemotherapy drugs achieve their therapeutic effect by acting upon different targets or different pathways. Therefore, Bliss Independence supports the clinical underlying assumption of independent drug action in combination chemotherapies. Although Bliss Independence supports the clinical underlying assumption of independent drug action, it does not entirely account for the sources of non-independence in drug resistance (**Fig. 18 A**).

As demonstrated by the results of this research project, independent action may not be entirely sufficient in explaining the efficacy of combination chemotherapy, especially in the cases where combinations performed worse than expected. The results from this project also demonstrated that the majority of combination chemotherapy drugs perform worse than anticipated based on independent action. Although this outcome is disappointing, these findings

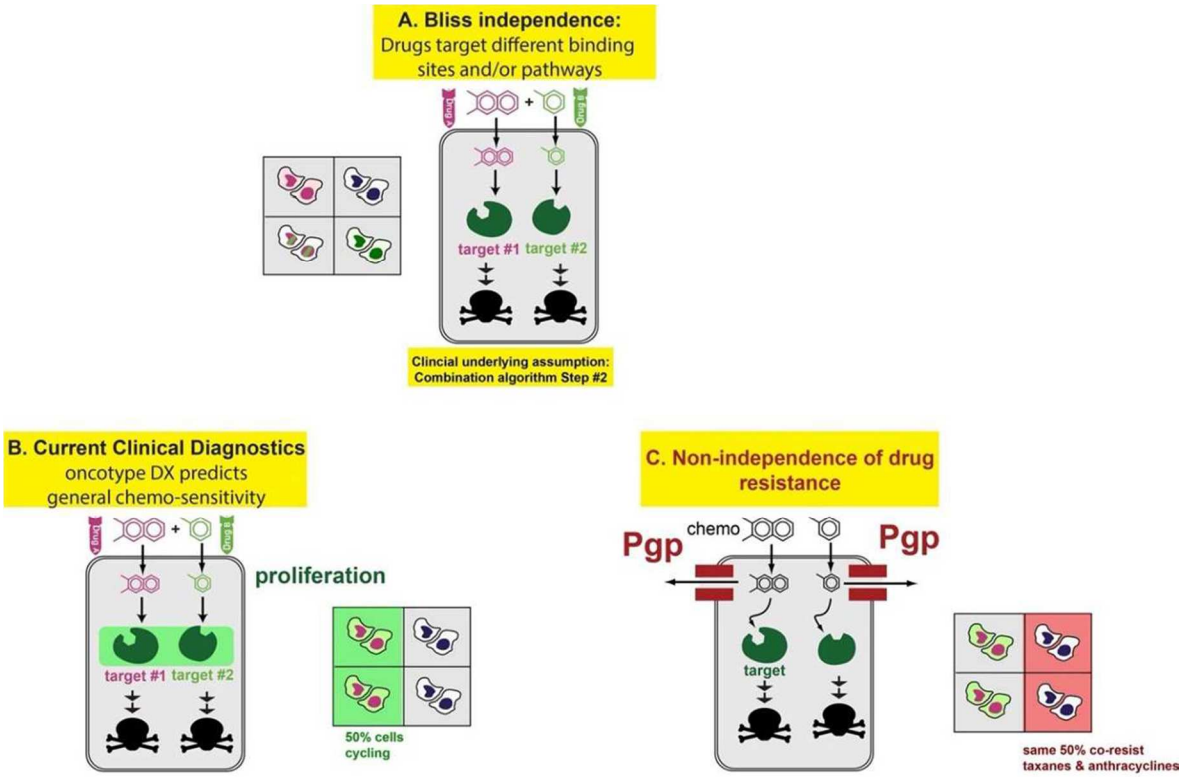


Figure 18. A. Bliss independence demonstrates the underlying assumption of the fractional kill hypothesis. The idea of independent action explaining clinical benefit is challenged by: **B.** Current clinical diagnostic techniques that determine tumor chemosensitivity and by: **C.** Drug resistance can arise from drug efflux pumps such as P-glycoprotein (Pgp) that can pump out multiple different substrates simultaneously.

are supported by existing cancer diagnostic techniques, such as Oncotype DX. Oncotype DX is diagnostic tool used for predicting breast cancer chemosensitivity based on proliferative markers. Characterizing tumor proliferation provides insight into a tumor's aggressiveness and risk for recurrence. This information can then be used to predict how a proliferative tumor may respond to a specific type of treatment. Using a combination of chemotherapy drugs that specific target the tumor within the proliferative stage of the cell cycle would contradict the notion of Bliss independence which assumes drugs within a given combination have different mechanisms of action (**Fig. 18 B**). Tumors that respond worse to chemotherapy than expected can be attributed to mechanisms of drug resistance. For example, if a tumor that highly expresses Pgp is being treated with a combination anthracycline and taxane drugs, it can be

predicted that treatment will be minimally effective since both drugs are substrates of Pgp, and ultimately are pumped out of the tumor cells (**Fig. 18 C**). Co-resistant drug mechanisms often arise from acquired resistance after first-line treatment, and therefore, is not assessed within the scope of this specific research project but is explored in other aspects of the Douglass lab.

Ongoing experimental work aims to characterize these sources of drug resistance, specifically by experimentally observing the effects of Pgp across different tumor cell lines and in the presence of different classes of chemotherapy drugs. Through experimental techniques such as the RealTime-Glo Cell Viability Assay, fluorescent microscopy, and flow cytometry, the experimentalists in the Douglass lab are working to characterize how Pgp is involved in multidrug resistance, in which one cancer is resistant to two drugs simultaneously. This thesis work served to provide clinically supported combination chemotherapy response rate data to serve as a guidance for future work in exploring more effective combination chemotherapy options and for overcoming multidrug resistance.

REFERENCES

1. Understanding What Cancer Is: Ancient Times to Present. Accessed February 28, 2022. https://www.cancer.org/cancer/cancer-basics/history-of-cancer/what-is-cancer.html
2. Karamanou M, Diamadis A, Androutsos G. The first cases of cancer in antiquity. *J BUON*. 2008;13(4):601-608.
3. Faguet GB. A brief history of cancer: Age-old milestones underlying our current knowledge database. *International Journal of Cancer*. 2015;136(9):2022-2036. doi:10.1002/ijc.29134
4. Casás-Selves M, DeGregori J. How cancer shapes evolution, and how evolution shapes cancer. *Evolution (N Y)*. 2011;4(4):624-634. doi:10.1007/s12052-011-0373-y
5. National Center for Health Statistics. Health, United States, 2019: Table 004. Hyattsville, MD. 2021. Available from: https://www.cdc.gov/nchs/hus/contents2019.htm. Published online 2019:4.
6. Crimmins EM. Lifespan and Healthspan: Past, Present, and Promise. *Gerontologist*. 2015;55(6):901-911. doi:10.1093/geront/gnv130
7. A note from history: Landmarks in history of cancer, part 3 - Hajdu - 2012 - Cancer - Wiley Online Library. Accessed March 9, 2022. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/cncr.26320
8. Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. *J Canc Sci Ther*. 2009;01(02): i-iv. doi:10.4172/1948-5956.100000e2
9. Plesca M, Bordea C, El Houcheimi B, Ichim E, Blidaru A. Evolution of radical mastectomy for breast cancer. *J Med Life*. 2016;9(2):183-186.
10. Röntgen WC. Ueber eine neue Art von Strahlen. *Annalen der Physik*. 1898;300(1):12-17.

doi:10.1002/andp.18983000103

11. Coutard H. PRINCIPLES OF X RAY THERAPY OF MALIGNANT DISEASES. *The Lancet*. 1934;224(5784):1-8. doi:10.1016/S0140-6736(00)90085-0
12. Gianfaldoni S, Gianfaldoni R, Wollina U, Lotti J, Tchernev G, Lotti T. An Overview on Radiotherapy: From Its History to Its Current Applications in Dermatology. *Open Access Maced J Med Sci*. 2017;5(4):521-525. doi:10.3889/oamjms.2017.122
13. Grubbé EH. Priority in the Therapeutic Use of X-rays. *Radiology*. 1933;21(2):156-162. doi:10.1148/21.2.156
14. Hajdu SI, Darvishian F. A note from history: Landmarks in history of cancer, part 5. *Cancer*. 2013;119(8):1450-1466. doi:10.1002/cncr.27889
15. Silverberg E, Holleb AI. Major trends in cancer:25 year survey. *CA: A Cancer Journal for Clinicians*. 1975;25(1):2-7. doi:10.3322/canjclin.25.1.2
16. History of Cancer Treatments: Chemotherapy. Accessed March 19, 2022. https://www.cancer.org/cancer/cancer-basics/history-of-cancer/cancer-treatment-chemo.html
17. Temporary Remissions in Acute Leukemia in Children Produced by Folic Acid Antagonist, 4-Aminopteroyl-Glutamic Acid (Aminopterin) | NEJM. Accessed March 19, 2022. https://www.nejm.org/doi/10.1056/NEJM194806032382301?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed
18. Effect of Methotrexate Therapy upon Choriocarcinoma and Chorioadenoma - Min Chiu Li, Roy Hertz, Donald B. Spencer, 1956. Accessed March 19, 2022. https://journals.sagepub.com/doi/abs/10.3181/00379727-93-22757
19. Treatment of Pulmonary Tuberculosis with Streptomycin and Para-Amino-Salicylic Acid. *Br Med J*. 1950;2(4688):1073-1085.

20. Law LW. Effects of combinations of antileukemic agents on an acute lymphocytic leukemia of mice. *Cancer Res*. 1952;12(12):871-878.

21. Milestone (1955): Creation of CCNSC. Accessed March 19, 2022. https://dtp.cancer.gov/timeline/flash/milestones/M3_CCNSC.htm

22. M.D VTD Jr, DeVita-Raeburn E. *The Death of Cancer: After Fifty Years on the Front Lines of Medicine, a Pioneering Oncologist Reveals Why the War on Cancer Is Winnable--and How We Can Get There*. Farrar, Straus, and Giroux; 2015.

23. Alkylating Agents. In: *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Accessed March 19, 2022. http://www.ncbi.nlm.nih.gov/books/NBK547849/

24. Venkatesh P, Kasi A. Anthracyclines. In: *StatPearls*. StatPearls Publishing; 2022. Accessed March 19, 2022. http://www.ncbi.nlm.nih.gov/books/NBK538187/

25. Skipper HE, Schabel FM, Mellett LB, et al. Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. *Cancer Chemother Rep*. 1970;54(6):431-450.

26. Devita VT Jr, Serpick AA, Carbone PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Intern Med*. 1970;73(6):881-895. doi:10.7326/0003-4819-73-6-881

27. National Cancer Act of 1971 - National Cancer Institute. Published February 16, 2016. Accessed March 19, 2022. https://www.cancer.gov/about-nci/overview/history/national-cancer-act-1971

28. Alam N. Impact of 1971's National Cancer Act Marked. NIH Record. Published December 10, 2021. Accessed March 19, 2022. https://nihrecord.nih.gov/2021/12/10/impact-1971s-national-cancer-act-marked

29. Xue Y, Wilcox WR. Changing paradigm of cancer therapy: precision medicine by next-generation sequencing. *Cancer Biol Med*. 2016;13(1):12-18. doi:10.28092/j.issn.2095-3941.2016.0003

30. Padma VV. An overview of targeted cancer therapy. *Biomedicine (Taipei)*. 2015;5(4):19. doi:10.7603/s40681-015-0019-4

31. Mazzotta M, Krasniqi E, Barchiesi G, et al. Long-Term Safety and Real-World Effectiveness of Trastuzumab in Breast Cancer. *J Clin Med*. 2019;8(2):254. doi:10.3390/jcm8020254

32. Blank PR, Dedes KJ, Szucs TD. Cost-Effectiveness of Cytotoxic and Targeted Therapy for Metastatic Breast Cancer: A Critical and Systematic Review. :27.

33. Fundytus A, Sengar M, Lombe D, et al. Access to cancer medicines deemed essential by oncologists in 82 countries: an international, cross-sectional survey. *The Lancet Oncology*. 2021;22(10):1367-1377. doi:10.1016/S1470-2045(21)00463-0

34. Research C for DE and. Frequently Asked Questions on Patents and Exclusivity. *FDA*. Published online February 22, 2022. Accessed February 28, 2022. https://www.fda.gov/drugs/development-approval-process-drugs/frequently-asked-questions-patents-and-exclusivity

35. WHO Expert Committee on the Selection of Essential Drugs, Organization WH. *The selection of essential drugs: report of a WHO expert committee [meeting held in Geneva from 17 to 21 October 1977]*. World Health Organization; 1977. Accessed March 19, 2022. https://apps.who.int/iris/handle/10665/41272

36. Dusetzina SB. Drug Pricing Trends for Orally Administered Anticancer Medications Reimbursed by Commercial Health Plans, 2000-2014. *JAMA Oncology*. 2016;2(7):960-961. doi:10.1001/jamaoncol.2016.0648

37. Yabroff KR, Zhao J, de Moor JS, et al. Factors Associated With Oncologist Discussions of the Costs of Genomic Testing and Related Treatments. *JNCI: Journal of the National Cancer Institute*. 2020;112(5):498-506. doi:10.1093/jnci/djz173

38. Cortes J, Perez-García JM, Llombart-Cussac A, et al. Enhancing global access to cancer medicines. *CA: A Cancer Journal for Clinicians*. 2020;70(2):105-124. doi:10.3322/caac.21597

39. Cheung WY, Kornelsen EA, Mittmann N, et al. The economic impact of the transition from branded to generic oncology drugs. *Curr Oncol*. 2019;26(2):89-93. doi:10.3747/co.26.4395

40. Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics. Published online 2018:19.

41. Hess LM, Brnabic A, Mason O, Lee P, Barker S. Relationship between Progression-free Survival and Overall Survival in Randomized Clinical Trials of Targeted and Biologic Agents in Oncology. *J Cancer*. 2019;10(16):3717-3727. doi:10.7150/jca.32205

42. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment*. World Health Organization; 1979. Accessed March 19, 2022. https://apps.who.int/iris/handle/10665/37200

43. RECIST. Accessed March 1, 2022. https://recist.eortc.org/

44. Aykan NF, Özatlı T. Objective response rate assessment in oncology: Current situation and future expectations. *World J Clin Oncol*. 2020;11(2):53-73. doi:10.5306/wjco.v11.i2.53

45. Thiesse P, Ollivier L, Di Stefano-Louineau D, et al. Response rate accuracy in oncology trials: reasons for interobserver variability. Groupe Français d'Immunothérapie of the Fédération Nationale des Centres de Lutte Contre le Cancer. *J Clin Oncol*. 1997;15(12):3507-3514. doi:10.1200/JCO.1997.15.12.3507

46. Jang GS, Kim MJ, Ha HI, et al. Comparison of RECIST version 1.0 and 1.1 in assessment of tumor response by computed tomography in advanced gastric cancer. *Chin J Cancer Res*. 2013;25(6):689-694. doi:10.3978/j.issn.1000-9604.2013.11.09

47. Choi JH, Ahn MJ, Rhim HC, et al. Comparison of WHO and RECIST criteria for response in metastatic colorectal carcinoma. *Cancer Res Treat*. 2005;37(5):290-293. doi:10.4143/crt.2005.37.5.290

48. Trillet-Lenoir V, Freyer G, Kaemmerlen P, et al. Assessment of tumour response to chemotherapy for metastatic colorectal cancer: accuracy of the RECIST criteria. *BJR*. 2002;75(899):903-908. doi:10.1259/bjr.75.899.750903

49. Park JO, Lee SI, Song SY, et al. Measuring Response in Solid Tumors: Comparison of RECIST and WHO Response Criteria. *Japanese Journal of Clinical Oncology*. 2003;33(10):533-537. doi:10.1093/jjco/hyg093

50. Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK. Systems biology, and combination therapy in the quest for clinical efficacy. *Nat Chem Biol*. 2006;2(9):458-466. doi:10.1038/nchembio817

51. Palmer AC, Sorger PK. Combination Cancer Therapy Can Confer Benefit via Patient-to-Patient Variability without Drug Additivity or Synergy. *Cell*. 2017;171(7):1678-1691.e13. doi:10.1016/j.cell.2017.11.009

52. Plana D, Palmer AC, Sorger PK. Independent Drug Action in Combination Therapy: Implications for Precision Oncology. *Cancer Discovery*. 2022;12(3):606-624. doi:10.1158/2159-8290.CD-21-0212

53. Livingston RB. *Single Agents in Cancer Chemotherapy*. Springer Science & Business Media; 2012.

54. SEER*Explorer. Accessed March 19, 2022. https://seer.cancer.gov/explorer/index.html

55. Warner JL, Cowan AJ, Hall AC, Yang PC. HemOnc.org: A Collaborative Online Knowledge Platform for Oncology Professionals. *J Oncol Pract*. 2015;11(3):e336-e350. doi:10.1200/JOP.2014.001511

56. Grappling with the Data Explosion in Oncology. *Oncology & Hematology Review,*. Published online November 19, 2015. Accessed February 25, 2022. https://touchoncology.com/diagnostics-and-screening/journal-articles/grappling-with-the-data-explosion-in-oncology/

57. Pokhriyal R, Hariprasad R, Kumar L, Hariprasad G. Chemotherapy Resistance in Advanced Ovarian Cancer Patients. *Biomark Cancer*. 2019;11:1179299X19860815. doi:10.1177/1179299X19860815

58. Falzone L, Salomone S, Libra M. Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. *Front Pharmacol*. 2018;9:1300. doi:10.3389/fphar.2018.01300

59. Hanahan D, Weinberg RA. The Hallmarks of Cancer. *Cell*. 2000;100(1):57-70. doi:10.1016/S0092-8674(00)81683-9

60. An Update on Cancer Deaths in the United States. Centers for Disease Control and Prevention. Published February 28, 2022. Accessed March 9, 2022. **https://www.cdc.gov/cancer/dcpc/research/update-on-cancer-deaths/index.htm**

61. Cancer Facts & Figures 2022. Published online 1930:80.

62. Murphy SL, Kochanek KD, Xu JQ, Arias E. Mortality in the United States, 2020. NCHS Data Brief, no 427. Hyattsville, MD: National Center for Health Statistics. 2021. DOI: https://dx.doi.org/10.15620/cdc:112079external icon. doi:10.15620/cdc:112079

63. Connor AA, Gallinger S. Pancreatic cancer evolution and heterogeneity: integrating omics and clinical data. *Nat Rev Cancer*. 2022;22(3):131-142. doi:10.1038/s41568-021-00418-1

64. The top 10 causes of death. Accessed March 19, 2022. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

65. Gottesman MM. Mechanisms of Cancer Drug Resistance. *Annual Review of Medicine*. 2002;53(1):615-627. doi: 10.1146/annurev.med.53.082901.103929

66. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer*. 2018;18(7):452-464. doi:10.1038/s41568-018-0005-8.

67. Hille S, Rein DT, Riffelmann M, et al. Anticancer drugs induce mdr1 gene expression in recurrent ovarian cancer. *Anti-Cancer Drugs*. 2006;17(9):1041-1044. doi:10.1097/01.cad.0000231480.07654.b5

68. Gottesman MM, Lavi O, Hall MD, Gillet JP. Toward a Better Understanding of the Complexity of Cancer Drug Resistance. *Annual Review of Pharmacology and Toxicology*. 2016;56(1):85-102. doi:10.1146/annurev-pharmtox-010715-103111

69. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov*. 2006;5(3):219-234. doi:10.1038/nrd1984

70. Rees DC, Johnson E, Lewinson O. ABC transporters: The power to change. *Nat Rev Mol Cell Biol*. 2009;10(3):218-227. doi:10.1038/nrm2646