Using Functional Genomics and Artificial Intelligence to Reverse Engineer Human Cancer Cells

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PREFACE

My overriding motivation in writing this book is a phrase that you will encounter several times in the pages to come. That phrase is: “There is no cure for metastatic disease”. The reason this phrase is so important is because, despite the enormous progress we have made over the decades from research on cancer, there has been very little progress for patients once their disease has spread to distant organs. And because of that, it is clearly time for us as a community to try some new approaches because standard chemotherapy, despite its uses in other phases of the disease, is not going to get us to the last mountaintop, the cure for metastatic cancer. In my opinion, one such strategy involves the application of modern artificial intelligence (AI) and machine learning (ML) methods to the mountains of genomic data that have been amassed from cancer patients and cancer-derived cell lines to develop strategies that can allow for truly personalized strategies for reverse engineering cancer in individual patients. The goal, therefore, of this book is to convince the reader that this is possible and, at the very least, an avenue worth pursuing. I should say at the outset that I will emphasize in this book how AI analysis of genomic data can lead us to better use of targeted cancer therapies. At the same time, other people are working on developing similar ways to use computational and AI methods to improve the use of immunotherapies for cancer, because immunotherapy provides another set of tools that can be used for patients with metastatic cancer. Since I am not an immunologist, I won’t discuss those approaches here as they can be found in other publications.

Because my goal is to convince you of something that you may not see as obvious or even feasible, I have devoted several chapters in the beginning of the book to building the case for my central thesis. To start this process, chapter 1 is aimed at setting the stage by summarizing how research on cancer over the decades has had a significant positive impact on public health. Indeed, it is precisely because we have come so far that I refer to the cure for metastatic disease as the last mountaintop in cancer research.

What I will ultimately propose in this book is a new approach for treating end stage or metastatic cancer. Thus, it is important for readers who are

not oncologists to have a basic understanding of how the traditional modes of cancer therapy work, and what their strengths and shortcomings are, and that is the subject of chapter 2.

As one progresses through this book it will become clear that a centerpiece of any novel strategy for metastatic cancer that does not involve chemotherapy or immunotherapy, will have to instead make better use of modern targeted cancer drugs. For that to make sense, one must have a basic understanding of the oncogenomic basis of cancer, and that is the subject of chapter 3. Cancer is, indeed, a genomic disease that is influenced by genetic factors and any strategy that will move us forward from where we are must use this fact as the starting point for better and more effective therapeutics.

As I mentioned above, we now have available to us massive amounts of genomic data from thousands of patients with cancer. In addition, we have amassed the same types of data from the hundreds of cancer cell lines that have been developed from biopsy tissues obtained from cancer patients. As I will argue throughout this book, the use of cell lines is absolutely necessary if we are going to put function to the otherwise descriptive genomic data that we have for patient-derived cancer tissues and cancer cells. Because of that necessity, chapter 4 is aimed at trying to convince the reader, and the skeptics, that cancer cell lines are excellent models of the specific disease that was experienced by the patients from which the cell lines were derived. There are some impressive and amazing organizations (The Broad Institute, the Wellcome Trust and others) that have invested millions of dollars to generate the kinds of functional genomics data that can only be obtained from cell lines, and we cannot ignore those data sets because of outmoded ideas about the usefulness of cancer cell lines. Thus, if you are one of those skeptics, you must read chapter 4. And even if you are one of the many thousands of cancer scientists around the world that use cancer cell lines in their work, you will also find some interesting tidbits in this chapter.

Chapter 5 is devoted to a description of how many groups and labs have used genome-scale essentiality screens in hundreds of cancer cell lines and how the data that has been obtained from these impressive experiments truly puts the function in functional genomics. The data derived from these kinds of experiments are what allows us to transform genomic data that are otherwise descriptive and noisy to data that are sharp and precise and, importantly, make predictions that are testable!

Thus, the first five chapters of this book are designed to set the stage for the data analysis sections that are presented in chapters 6 through 11. In these chapters I take a stepwise approach to the use of functional genomics for reverse engineering cancer cells. The first step is to use functional genomics to identify the genes that are most likely to be good targets of modern cancer drugs. Then, I examine how this approach can be used to determine, for those good genomic targets, the relationship between their essentialness and their sensitivity to drugs that target them. Next, I show how drug sensitivity data can be used as an alternative strategy to identify essential genes that are good drug targets.

The last three chapters of the book are aimed at tying this all together into what will be proposed as a novel strategy to reverse engineer metastatic cancer cells in patients. The ultimate strategy will include the application of Artificial Intelligence and Machine Learning methods to make predictions about drug sensitivity and resistance in individuals, and to leverage those predictions to design potent targeted drug combinations that can have high efficacy with little toxicity.

I wrap up the book with a reality check and a discussion of the paths that might be taken to implement the strategies ultimately developed as a result of this work so that effective clinical trials can be designed to provide the ultimate test of the methods and strategies that can come from developing reverse engineering strategies for metastatic cancer.

There is no cure for metastatic disease. Traditional methods involving chemotherapy alone have not worked. It is time for some alternative approaches.

ACKNOWLEDGEMENTS

In the nearly forty years that I have been working as a breast cancer researcher, I have had the opportunity and pleasure to work with many outstanding individuals. Among them are the many graduate students, medical students, post-doctoral fellows, and radiation oncology residents that have worked in my lab and whom I was proud to mentor. In addition, I've had the opportunity to work with and collaborate with some of the best cancer researchers in the world. It has been an honor for me to know and work with these fine scientists and clinicians. And over this span of forty years, I have published many papers with these individuals. The work presented in this monograph is my personal distillation of the topics and challenges that I consider to be most important and urgent in making future progress aimed at curing more patients with cancer. As such, there are data that are presented here that have not been published elsewhere, and I want to acknowledge the brilliant and talented people that were instrumental in generating the data shown in chapters 6 through 11.

I would first like to acknowledge Mr. Daniel Couch who brought his incredible skill and imagination to the generation of the MySQL database that underlies much of the work presented in these chapters. Daniel also wrote and launched all the data mining apps that are present on the SUM Breast Cancer Cell Line Knowledge Base (SLKBase). None of what has come later would have been possible without his work and talent.

Next, I'd like to acknowledge Mr. Kent Armeson. Kent is an outstanding biostatistician who wrote the R-scripts that resulted in the statistical analysis of the data shown as box and whisker plots and correlation plots shown in chapters 6 and 7.

I want to give a special acknowledgement to Dr. Steve Guest who worked with me for several years at the Karmanos Cancer Institute and the Medical University of South Carolina. Steve was the technical genius behind all of the genome-scale essentiality screens that we performed using the SUM breast cancer cell lines, among other cell lines. In addition, Steve worked with another brilliant computer scientist, Mr. Eric Czech, and together they performed the first-generation AI analysis of functional genomic data that resulted in a gene signature that can be used to predict

sensitivity to the targeted drug Navitoclax. Some of the data from that analysis are shown in chapter 10.

And finally, I want to acknowledge Drs. Brittany Ivey and Jammie Mills, not only for the great work they did while in my lab at MUSC, but also for their critical reading of the manuscript.

The idea was fantastically, wildly improbable. But like most fantastically, wildly improbable ideas it was at least as worthy of consideration as a more mundane one to which the facts had been strenuously bent to fit. “

—Douglas Adams, “The Long Dark Tea-Time of the Soul”

CHAPTER 1

THE IMPACT OF 50 YEARS OF CANCER RESEARCH ON CANCER INCIDENCE, AND SURVIVAL

The goal of this book is to discuss what I consider to be the last mountaintop to be conquered on the war on cancer; how to effectively deal with clinically detectable metastatic disease. It should be obvious to everyone by now that despite our best efforts to improve the efficacy of local therapies for cancer, and for developing adjuvant chemotherapeutic approaches that make the emergence of clinical metastatic disease less likely, too many people still die of their cancer, and when they do, it is almost always because they succumb to their metastatic disease.

A brief primer on carcinogenesis and cancer prevention

Before we delve deeply into how we might use our understanding of cancer biology to conquer this last mountaintop of cancer research and clinical oncology, it is worthwhile to devote a bit of space at the outset to a discussion of how our understanding of the process of cancer development and carcinogenesis has had an enormous impact on cancer prevalence and cancer mortality. During the early years of the war on cancer, there were many aspects of cancer research that were not focused on how to best diagnose and treat cancer in patients. Rather, there were decades of work that were focused on understanding the basic biology of cancer development, and knowledge gained from this work was used to improve public health by identifying the causes of cancer in humans and instituting programs to reduce the cancer burden of the population.

Some of the biggest successes that led to the declining incidence of cancer were the result of a deep understanding of the process of cancer development in humans; a process known as carcinogenesis. When research in carcinogenesis began in the late 1960's we had almost no idea of the kinds of agents that cause cancer, and as a result, we were essentially ignorant of the mechanisms of human carcinogenesis.

However, significant progress in this field was made in the subsequent twenty or so years after the start of the war on cancer and the discoveries that were made resulted in a complex and essentially complete understanding of the molecular mechanisms by which chemical carcinogens, radiation carcinogens, and viral carcinogens cause cancer in humans (Poirier 2018; Penning 2011; Upton 1986). While the majority of this book will deal with advances in the treatment of cancer once it is diagnosed, the impact of our understanding of carcinogens and their mechanisms of action on the world-wide cancer burden cannot be overstated, because it is precisely because of our deep understanding of the molecular mechanisms of carcinogenesis that preventative measures at both personal and industrial levels were implemented that have had a large public health impact. For example, watch dial painters once used radioactive Radium to make the dials glow in the dark. As a result of our understanding of the mechanisms of radiation carcinogenesis, workers like these are no longer exposed to radioactive radium, and thus the bone cancers that occurred in those workers is no longer an industrial health issue. More recently, the understanding of the role of chemicals known as aromatic amines in the genesis of bladder and other cancers resulted in changes in the dye industry making this process safer for workers in this field and the prevention of many cancers. A particular type of aromatic amine called Aflatoxin, is the product of the mold *Aspergillus flavus* which for many years contaminated peanut crops, especially in African countries, and resulted in the development of liver cancer. There are many similar examples that involve many types of cancer-causing agents that are either no longer on the market, or are highly regulated, and thousands of papers have been published on this topic. Perhaps the most significant and far-reaching translation of the understanding of the mechanisms of chemical carcinogenesis to public health was the complete elucidation of how chemicals in cigarette smoke cause the development and progression of lung cancer in humans.

During the 1980s and much of the 1990's a mountain of circumstantial evidence accumulated that linked exposure to cigarette smoke, either primarily or secondarily, to the development of lung cancer in people. However, because a direct and causal mechanistic link between specific agents in cigarette smoke and lung cancer had not been definitely established, tobacco companies were able to argue that the evidence linking tobacco smoke to cancer and other diseases was only correlative and therefore, possibly not correct. Thus, they continued to argue that their products were safe or at least, might be safe.

All of that came to an end upon the definitive establishment of the specific types of mutations caused in specific genes known to be important in lung cancer, and the definitive demonstration that chemicals in cigarette smoke directly caused those mutations. Those final pieces of evidence at the molecular and genomic levels, the true smoking gun as it were, transformed what was once circumstantial and correlative evidence into a definitive link between smoking and lung cancer. And while this evidence was sufficient to counter all arguments from tobacco companies about the dangers of smoking, the more tangible and immediate impact came from the ability to use such data to file multiple class action lawsuits against tobacco companies that resulted in fines and settlements in the many billions of dollars. Some of that money was used to compensate victims of smoking related lung cancer, but some of that money, ironically, was also used to fund additional cancer research. As a result of all of this, the entire culture associated with cigarette smoking changed dramatically in the United States, and this has, no doubt, saved countless lives. All one has to do is remember the days when people actually smoked on elevators, in airplanes, and restaurants to realize the culture shift that resulted from a deep understanding of the molecular mechanisms of carcinogenesis (Hatsukami 2022).

One final anecdote that further illustrates the importance of basic research in carcinogenesis to cancer prevention is illustrated by the elucidation of the role of the Human Papilloma Virus type 16 (HPV-16) in the development of cervical cancer in woman and now, squamous cancers of the head and neck in women and men. There have been decades of research on the molecular mechanisms by which certain viruses cause cancer in animals, and there have been a few Nobel prizes awarded as a result. Even though several types of cancers in animals can be caused by different types of viruses, it turns out that a viral etiology of cancer in humans is quite rare. But one exception to that rule is the HPV-16 virus which is one of the causes of cervical cancer and squamous cancer of the head and neck. In this case, once again, the mechanistic evidence is clear, and we have a deep understanding of how two specific viral onco-proteins (the HPV-16 E6 and E7 proteins) cause these cancers. This definitive understanding has led to the development of vaccines against the HPV-16 virus that are now commonly administered to adolescent boys and girls. The wide-spread availability of HPV-16 vaccines will have a dramatic impact on the prevalence of these important cancer types, and this in turn, will result in dramatic reductions in morbidity and mortality from these types of cancers (David Jenkins 2019).

Thus, because of the progress that has been made in elucidating the mechanisms of carcinogenesis at the molecular and genomic levels, the burdens of cancer have been reduced dramatically. It is also clear that, given the nature of how cancer develops and progresses (to be discussed in more detail later), it is much better to prevent cancer than to try and cure it. Thus, research in carcinogenesis can be considered a major success in the war against cancer.

Key fundamental aspects of cancer biology. Over my career as a cancer researcher, I have given many lectures to medical students, medical residents, graduate students, and post-doctoral fellows (and the occasional intrepid undergrad) on what I refer to as the *fundamentals of cancer biology*. I always begin my lectures by making clear what I consider to be one of the most fundamental and poorly appreciated aspects of the biology of cancer; that is the apparent "latency" of these diseases in people. By latency, I mean the time that elapses between when the process of cancer development begins and when the disease can actually be diagnosed by some type of medical procedure or even the detection or palpation of a lump or tumor. Invariably, students in my class are surprised to learn that this latent period in humans for blood cancers such as leukemia and lymphoma is a decade or longer, and the latent period for the development of solid cancers such as lung cancer, breast cancer, colon cancers, etc. is on the order of 20 to 30 years! That means that by the time a clinician makes a definitive diagnosis of cancer in a patient, the development of that cancer has been ongoing for over 20 years. Thus, we should be careful with the term "early detection" because even the earliest of detections still occurs relatively late in the process of disease development. The importance of understanding this fundamental aspect of cancer biology to the ultimate goal of curing metastatic disease will become clearer later in this book.

The second fact that is related to the idea of cancer latency that my students are often surprised to learn but that is essential if one is to understand the biology of cancer is that cancer is a clonal disease. What that means is that the disease that is diagnosed as cancer in a patient had its origins in a single "transformed" cell. By the time the disease is diagnosed, the neoplasm as it is called, consists of tens of millions of cells all of which are the descendants of that single transformed cell. During the evolution of cancer many sub-clones develop that contribute to the emergence of a complex and heterogeneous neoplasm that play an important role in how the cancer responds to therapy. As the disease progresses the millions of cells present at diagnosis can grow to become

over one billion cells, and once the disease progresses such that the tumor burden is on the order of one hundred billion cells, the patient will succumb to their disease. So that then, is the ultimate challenge of the Clinical Oncologist: first, how to detect cancer at its earliest stages when relatively fewer cancer cells are present, then how to eradicate as many of those few million cells as possible after the initial diagnosis, and then, how to prevent any residual cells from continuing to grow and proliferate to a point where the tumor burden becomes too great to be consistent with life. The process of cancer development from a single transformed cell to a few million cells at diagnosis, to billions of cells late in disease progression takes a long time, and a lot of biology happens during those years, all of which are important to the ultimate development of metastatic disease.

The stages of cancer progression. There have been many articles and books written on the biology of cancer progression and a detailed accounting of this process is beyond the scope of this book (for example see: (Coppola 2010)). However, before we delve into the main focus of the book, which is how to best leverage our understanding of cancer at the molecular level to develop 21st century treatment strategies ultimately aimed at curing metastatic disease, it's helpful at this point to say a few words about all of the biology that goes on during the so-called latent period between the time of the first transformed cell to the diagnosis of cancer. For many types of cancer, we can identify discreet stages of disease that occur before the cells of the neoplasm acquire full malignant potential. That statement on its own indicates that cancer in its earliest stages is not the life-threatening disease that it evolves to become. So then, what are some of the characteristics that the descendants of the first transformed cell acquire on their way to becoming fully malignant cells that can kill their host?

There are several, what we refer to as, hallmarks of cancer (Hanahan and Weinberg 2011). These hallmarks represent distinct biological features that cells acquire during their evolution to become cancer. For brevity's sake, I will list them here, but keep in mind, that during the process of cancer evolution, cells can acquire these features in any order, though one of them in particular (genomic instability) is important for the acquisition of the others and for the emergence of sub-clones within the neoplasm.

- Cellular immortality. Yes, cancer cells, unlike your normal cells can live forever.
- Independence of proliferative signals from hormones or growth factors

- Resistance to growth inhibitory signals
- Resistance to signals that would induce programmed cell death
- Ability to evade immune surveillance
- Acquisition of genomic instability
- Acquisition of cellular motility
- Acquisition of ability to invade surrounding normal healthy tissue
- Ability to enter and exit the vasculature and proliferate in new sites

Early in the evolution of a malignant neoplasm, cells might exhibit only a few of these hallmarks and this is one of the reasons why early detection can be so important, because once cells acquire all of these hallmarks and become fully malignant and metastatic, clinical treatment becomes more difficult and sometimes impossible. And, as I indicated above, the acquisition of these properties takes time and occurs in discreet steps that are the result of specific alterations in the genome of the cancer cells.

Now, with that as a background, let's now begin a consideration of how understanding cancer biology plays a role in cancer therapy, both traditionally and in the future.

CHAPTER 2

THE THREE ARMS OF TRADITIONAL CLINICAL ONCOLOGY

Now that we have a better understanding of what patients and oncologists are dealing with once the diagnosis of cancer is made, I will provide a brief overview of the strategies that are used to combat the disease. Before that though, we need to introduce one more fundamental aspect of the biology of human cancer, and that has to do with what we refer to as cancer progression.

We have established that cancer originates in a single cell in a single tissue and is the result of many rounds of cell division that takes place over decades. That single transformed cell ultimately gives rise to a tumor or a lump that can be detected directly or by some of our newest imaging techniques (X-rays, ultrasounds, CT scans, MRI scans, etc.) The lump or tumor that is detected is referred to as the *primary tumor* or as *local disease* because it is diagnosed in the same tissue that was the home of the original transformed cell. Thus, a single transformed cell in one of the milk ducts of breast tissue can give rise to a tumor that is detected in the breast. The same is true for transformed cells in lung tissue giving rise to a lung tumor and transformed cells in the colon giving rise to a colon polyp and then a colon cancer. So, once again, the primary tumor is what is referred to as local disease. If local disease is all we had to deal with, cancer would not be the clinical problem that it is. Indeed, a benign tumor is one that develops locally and remains in the local region throughout its natural history because the cells of a benign neoplasm do not have the capacity to invade normal tissue or the vasculature. As a result, it is rare for anyone to die of benign disease. The opposite of benign disease is malignant disease, and this is what we are referring to when we use the term cancer. The fundamental difference between benign and malignant disease is that malignant cancer cells acquire the ability to become motile and invasive. That is, the cells don't just stay put within the tumor they have formed, but rather, the cells burrow away from the primary tumor invading and destroying the normal cells along the way. And as part of

that process, these malignant cancer cells worm their way into the vasculature; the small blood vessels and lymphatic vessels that are, no pun intended, the lifeblood of every living tissue. Once these malignant cancer cells gain access to the vasculature, the cells can be rapidly distributed throughout the body. Then, sometimes the cells worm their way back out of the circulation and into a tissue far distant from the primary tumor. This dissemination of cancer cells and the establishment of secondary tumors at sites far distant from the primary tumor is the process of metastasis. As I have already mentioned and will be discussed in great detail later, dealing with metastatic cancer is the ultimate challenge in clinical oncology. Thus, finding novel ways to cure metastatic disease is, therefore, the last mountaintop in clinical oncology and it is a challenge that remains to be conquered.

Given our general understanding of the progression of human cancer, let's return to the goals of the three arms of Oncology which are: surgical oncology, radiation oncology, and medical oncology.

Surgical Oncology. As we discussed above, a primary tumor that is detected either by the patient or their doctor usually consists of from 1 million cells to about 10 million cells and sometimes more depending on the size of the primary tumor. In Clinical Oncology it is well known that size does indeed matter. The smaller the tumor that is first detected and treated the better the outcome of the patient, and this makes sense in terms of how cancer progresses. So, the first line of attack in the clinical battle against cancer almost always involves a surgical oncologist whose goal is to remove as many of those cancer cells as possible using the most modern methods of surgical oncology. As a friend and former colleague of mine used to say, 'a chance to cut is a chance to cure'. He is obviously a surgeon.

Keep in mind that when the surgeon removes a primary tumor, he or she can see the lump itself and they can see what they consider to be the margins of that lump, but they can't see the cancer cells themselves. Thus, the goal of any surgical procedure for cancer is to remove the entire primary tumor and obtain "clear margins", i.e., edges free of cancer cells. It is the job of the surgical pathologist to examine the tumor once it's removed and determine if indeed the margins are clear of cancer cells. This is critical because once again, it is well known that if the surgeon truly "gets it all" the patient is cured of their disease. Full stop. And it follows then that the smaller the tumor is, the more likely it is that the surgical procedure removes every last cancer cell from a patient's body.

As a result of decades of clinical research, we have a very good understanding of the outcomes that can be expected for many types of cancer following a surgical intervention. And we know that in some cases, particularly those in which the primary tumor is large or for which the surgery is difficult and complex, e.g., the Whipple procedure for pancreas cancer, if nothing else is done beyond the surgery, some patients will recure with the same cancer at the primary site either months or years later, and this is never a good outcome. Indeed, these so-called local recurrences can occur even in patients who had clear margins after the primary surgery, although this is less common.

Radiation Oncology. Enter the radiation oncologist. The field of radiation oncology is highly complex and makes use of cutting-edge technology in terms of radiation beam energy and design, and real-time imaging of the tumor being treated. Although in some case, radiation treatment is the primary modality for the treatment of primary cancers, for the purposes of this discussion, I want to focus on the role of the radiation oncologist as a partner to the surgical oncologist in achieving so-called "local control" of a primary tumor. Although there are now many examples of how this clinical partnership has had a great impact on cure rates for cancer, the specific example of how these modalities are used to treat breast cancer is illustrative of the broader field.

It wasn't very long ago that the standard surgical procedure for a woman (or a man) diagnosed with breast cancer was the modified radical mastectomy; a procedure where the entire breast was removed along with regional lymph nodes. This was, indeed, a radical strategy aimed at ensuring that the entire primary tumor was removed from the patient. As a result, local recurrence rates following this procedure were quite low. Over the years, the field of surgical oncology has moved toward the use of less radical and more conservative procedures and for breast cancer, the current standard of care is to perform a lumpectomy instead of a modified radical mastectomy, whenever possible. This procedure, which strives to remove only the tumor and leave as much of the breast intact as possible, yields a better cosmetic result and has been a factor in women being more amenable to early detection strategies like mammography. However, clinical research in this area demonstrated quite convincingly that, in terms of local control, i.e., the rate of local recurrence after surgery, lumpectomy was not as effective as the modified radical mastectomy. Local recurrence rates following lumpectomy alone were unacceptably high, which puts patients at higher risk of distant metastasis later in life. So, as I indicated above, this is where the radiation oncologist enters the picture.

The finding of unacceptably high local recurrence rates following lumpectomy alone meant that, despite best efforts to obtain clear margins, some residual cancer cells are left behind. Over time, these cells can grow into a new tumor at the primary site. In an attempt to combat this and retain lumpectomy as a primary strategy, studies were conducted to determine if administration of local radiation at the primary site after lumpectomy could reduce rates of local recurrence. The idea is that, following surgery with a course of radiation will kill any residual cancer cells left behind by the surgeon, and thus reduce or eliminate local recurrence. The clinical studies that examined the efficacy of lumpectomy followed by radiation were highly successful, and we now know that lumpectomy plus radiation is just as effective at achieving local control of breast cancer as modified radical mastectomy. This was a major advance in the treatment of breast cancer, which is the second most common cancer in women (Speers and Pierce 2016).

In an interesting twist on the success of this dual modality treatment, it was found that for some patients, the size of the primary breast cancer is too large to make lumpectomy feasible. For these patients, radiation therapy is sometimes given first to shrink the tumor and make it amenable to lumpectomy instead of the more radical surgery.

Over the years, there has been an increasing trend for more and more conservative surgeries for many types of cancer and in these cases, the combination of conservative surgery plus radiation therapy has served to optimize local control rates and minimize the rates of local recurrence. This is a great success story in clinical oncology.

Why then, do these state-of-the-art methods for eradicating the primary cancer not result in more cures of disease? The reason for this is because for those patients that don't recur at the site of the primary tumor, they can recur at distant sites, because as we discussed earlier, malignant cancer cells have that ability to invade the vasculature and travel to distant sites in the body. So, when a patient is treated locally with surgery plus radiation and recurs months or years later at one or more *distant* sites, that means that *at the time of surgery*, some of those cancer cells had *already moved away* from the primary tumor and were lurking undetected in other tissues of the body. Keep in mind, that all patients that are treated to achieve local control also undergo extensive imaging and screening to search for the presence of secondary tumors at distant sites. Most often, the results of these tests are negative. This means that from a clinical perspective, patients who complete their local therapy and have been screened for

distant disease are considered to be "clinically disease free". However, we know that some of these patients are not completely disease free because some fraction of them will recur at distant sites months or years later (as late as 15 years in some cases!).

Recall our discussion on the diagnosis of primary cancers. We said that primary tumors, when detected, usually consist of one-million cancer cells or more. Why can't we detect cancer at earlier stages, i.e., when there are fewer cancer cells? For starters, such a small tumor doesn't usually cause any symptoms, so patients don't know they have them. Secondly, our detection methods are not sensitive enough to know when a patient has only a few hundred or a few thousand cancer cells in their body. So, when patients who are considered to be clinically disease free after their primary cancer treatment recur at distant sites later, it's because there were a small number of cancer cells lurking at one or more of these distant sites, and they too went undetected until they grew into tumors of a detectable size, or when they caused symptoms. Such patients are said to have had "occult metastatic disease".

In situations like this, we can't determine which specific patients have occult metastatic disease, but we do have a good idea of the *likelihood* that a patient has such occult disease. Knowing the odds that a patient who is clinically disease free actually has occult metastatic disease can be used to make a determination of the potential for benefit of chemotherapy following the treatment of the primary tumor.

Medical Oncology. Enter the Medical Oncologist. The primary goal of the medical oncologist is to attack, what we refer to as, systemic disease. Systemic disease is the opposite of local disease. Systemic disease is the result of the process of metastasis in which the entire body *system* is at risk for the emergence of metastatic cancer. The tool used by medical oncologists to treat systemic disease is primarily chemotherapy.

Over the decades, medicinal chemists, pharmaceutical companies, and medical oncologists have developed a dizzying array of chemotherapeutic drugs. Often these drugs are used in combinations that have been painstakingly tested in clinical trials before being administered to patients. For the purposes of this discussion, it's important that people understand that most chemotherapeutic drugs are blunt instruments. That is, they are designed primarily to kill proliferating cells and not necessarily cancer cells. Since one of the defining characteristics of cancer cells is their propensity to undergo cell division (proliferation) in an uncoordinated and

unregulated manner, chemotherapeutic drugs are excellent at killing cancer cells. The problem is that cancer cells are not the only cells in the body that are proliferating. For example, the bone marrow is full of rapidly dividing cells whose job it is to consistently replenish the supply of red and white blood cells found in the circulation. Similarly, there are millions of proliferating cells lining the intestines whose job it is to continuously replenish the cells that are vital to the maintenance of the gastro-intestinal track, and to be sure that nutrients and water are absorbed from foods to be used as the energy that fuels life. The skin is another organ that is constantly turning over (hence house dust!) and thus the basal later (bottom) of the epidermis consists of rapidly proliferating cells.

As I mentioned above, chemotherapeutic drugs are blunt instruments and that means that these drugs are effective at killing all proliferating cells, not just cancer cells. It is this non-specificity that is the basis for the common side effects that are associated with chemotherapy; hair loss; nausea, vomiting and diarrhea, and various types of anemia. Thus, the medical oncologist is always trying to use chemotherapeutic drugs to kill as many cancer cells as possible without killing the patient with the drugs. Indeed, a large part of the training of medical oncologists revolves around the medical practices used to treat the toxicities that are caused by the chemotherapeutic drugs, and there have been great advances in these treatments. In the cases where there are a relatively few metastatic cancer cells in the body, chemotherapy can effectively kill essentially all the cancer cells without doing too much harm to the patient. In these cases, chemotherapy is curative. We know this because measurements of the overall survival rates in groups of patients who receive this type of chemotherapy are better than for those that don't. More often than not though, chemotherapy reduces tumor burden without eradicating all of the cancer cells and in these cases, the patients recur at distant sites months or years after their chemotherapy. Often, patients will recur later than they would have, had they not received chemotherapy, and thus, the chemotherapy can add years of life for the patient. But such patients are not cured.

Given that recurrence is possible following chemotherapy, I want to get back to the course of cancer treatment that was discussed above and now highlight the role of the medical oncologist in affecting cures for patients. Above, I described a scenario where a patient was treated with surgery and radiation to achieve local control of the cancer, and in which screening for overt metastatic disease indicated that the patient was clinically disease free. Now, imagine that this is a situation in which the medical oncologist

knows, based on years of clinical trial data for patients with similar characteristics of the primary tumor, that the patient has a 25% chance of having occult metastatic disease, even though it is not presently detectable. What does the medical oncologist and the patient do with this information?

In modern medical oncology, when these situations exist, and they are common, the standard of care is often to treat *all* the patients with a chemotherapeutic regimen that is considered "well tolerated" by most patients. This type of treatment is referred to as *adjuvant* (meaning added or additional) chemotherapy. One can see right away the advantages and disadvantages of this approach. The advantage is that by treating patients who have a relatively small number of cancer cells lurking somewhere in the body, the chances of killing all those remaining cancer cells without undue harm to the patient are relatively high. And indeed, adjuvant chemotherapy has contributed to improved *cure* rates for many types of cancer. The disadvantage of this strategy is that, in this scenario, 75% of the patients with similar types of disease will receive chemotherapy even though they don't have *any* occult metastatic cells lurking in their bodies. Thus, these patients were actually cured by their local therapy, but there was no definitive way to know this. Thus, they receive no benefit from the chemotherapy, but they will incur all the side effects. For the majority of patients who receive a cancer diagnosis, this is a gamble they are willing to take. As a result, this type of adjuvant chemotherapy has become the standard of care for many types of cancer because the consequences of recurring with full blown metastatic disease are so dire.

One of the most exciting areas of research in medical oncology today has the goal of developing more sophisticated ways to identify the specific patients that are most likely to have occult metastatic disease, and which are therefore most likely to benefit from the chemotherapy. There are already some excellent examples of strategies that are being used effectively to spare patients from chemotherapy who don't require it (Curtit et al. 2017; Markopoulos 2013; Carlson and Roth 2013). Increasingly, clinicians are making use of genome sequencing methods to examine circulating DNA in the blood of cancer patients, as detecting genomic alterations present in the cancer cells in the blood can be an early sign of the presence of occult metastatic cells. There are also new genomic strategies that make predictions about the likelihood of recurrence based on the genomic features present in the primary cancer. These genomic signatures are being used today to spare many patients from chemotherapy that they don't require.

There is no cure for metastatic disease

Earlier we used breast cancer treatment as an example of how surgery and radiation therapy have been used to virtually eliminate local recurrences of breast cancer. And yet, even though most women who are diagnosed with breast cancer do not die of their disease, too many women still do. Why? The reason is that when breast cancer cells disseminate throughout the body they go to places like the bone, the liver, and the brain. And in so doing, these metastatic cells can develop into dozens to hundreds of individual metastatic nodules. As these *secondary* tumors grow, they destroy the normal cells that are present in that secondary site. The liver is a remarkable organ, and we can live with only about half of our liver. Thus, breast cancer cells metastatic to liver can grow for a long time before they cause symptoms associated with liver disease. When these cancer cells destroy the function of more than half of the liver, the patient will die. The same is true for loss of function of brain tissue and bone marrow. In fact, it is the proliferation of metastatic breast cancer cells in bone that is the cause of so much pain endured by breast cancer patients once these metastases are established in bone and bone marrow.

So now you see the problem. Because once patients present with *clinically detectable* metastatic cancer, they are essentially *not curable*. The term, 'there is no cure for metastatic disease' is *almost* as true today as it was 30 years ago. And thus, this is where we need to do much better and develop novel strategies to get us to the top of this last mountaintop.

Before we delve further into what I consider to be potentially viable strategies that can get us to this mountaintop, it's instructive to discuss how medical oncologists use chemotherapy to combat clinically detectable metastatic cancer currently because as we will see, these methods can be effective, but they also can contribute to the problem that we are trying to deal with, and sometimes make it worse.

Chemotherapy for overt metastatic disease

There are now several different types of imaging strategies that can be used by oncologists to not only detect the presence of secondary cancers at metastatic sites, but also accurately measure their size, their metabolic activity, and their growth rates. Thus, when oncologists use chemotherapy to treat patients with overt metastatic disease, they use these imaging methods to determine just how well or poorly the drugs being used are working. For the most common types of cancer, there are standard