Leukemia understanding and treatment: Where do we stand?

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The focus of our lab's research is to understand the onset and progression of leukemia. In this context we use multiple animal and human models to understand the progression of blood cancer and try to develop new drugs that can be used to treat leukemia. I am writing this article to share the insights I gained during my journey with society, because the progression of science is a collective effort and cannot be achieved just by one individual. I am thankful to the program "Sharing UW-Madison Postdoctoral Scholarly Research with Non-Science Audiences," sponsored by the Wisconsin Initiative for Science Literacy (WISL). This program, made possible by the dedication of the WISL staff, specifically Cayce Osborne, Elizabeth Reynolds and Professor Bassam Shakhashiri, is instrumental in fostering connections between scientific exploration and a wider audience. I highly appreciate the WISL initiative because it can provide a platform for non-science audiences to gain basic understanding of how research is conducted in a laboratory setting and motivate the younger generation to step into the fascinating field of science exploration.

Leukemia is a type of cancer that affects our blood cells. In 2023, at least one person died of leukemia every hour in the US. The overall 5-year survival rate of leukemia was around 69% in 2023. The survival rate can be improved if the disease can be diagnosed at an early stage. There are several causative factors for leukemia, but one of the main factors is a previous history of any kind of blood disorder.

Clonal hematopoiesis of intermediate potential (CHIP) is one such blood disorder, which can result in the expansion of our blood stem cells because of mutations. A mutation in this context is a change in our DNA (DNA is made up of four different types of small molecules called nucleotides, which are labelled as A, T, G and C). A patient with CHIP has about a 10% risk of developing leukemia. CHIP usually occurs in the elderly. However, a patient's advanced age limits the options available for cancer treatment, as the age of body increases, cells can't tolerate the side effects of chemotherapy.

One out of five CHIP patients dies of heart failure across the globe. I lost both my great-grandfather and grandfather to a heart attack. Both were suffering from different kinds of diseases, in one case blood cancer and in the other diabetes and were never diagnosed with any kind of heart-related issues before the onset of their respective diseases. As a kid, I was always intrigued by the fact that though physiologically different, their diseases culminated to pathologically the same risk (heart failure) and ultimately their demise. The treatment both received was for their primary diseases, which were cancer and diabetes, but rather than these diseases themselves, their

secondary complications were more lethal. These situations sparked my interest in learning more about the human body, specifically in relation to diabetes and cancer.

As I explored cancer more deeply, I was fascinated by the fact that, despite the many things that can go wrong in our body, our bodies' repair and combat mechanisms are so well developed that they can tackle most of these problems. For instance, if a particular cell needs to divide, the most important step is to duplicate its genetic material (DNA). Before it starts the process of duplication, a cell ensures that it has all the required raw materials, and once the process is completed, it even has a mechanism to proofread so that there is a minimum number of errors in the process of duplication. If the cells detect an error in the process, they try to abort the duplication and rectify the error (Fig 1). If rectification is not possible, the cells will undergo programmed cell death. The process of division is very tightly regulated by a network of unique and redundant complex molecules called proteins. Proteins are encoded by DNA and are the building blocks of our body. They are required to maintain different body structures like skin, hair etc., and perform many important functions in our bodies.

At any given point of time, there will be several million cells undergoing division in our body. So, one can imagine the amount of work our body needs to do to maintain this regulation as tightly as possible. Just a peripheral glance at the pathways involved in regulation of cell division can seem very chaotic; however, there is an intricate order for this seemingly chaotic system, which has yet to be completely explored. Any changes to this order would manifest as diseases, and in this context, it can lead to the development of cancer.

When I first started learning about cancer in my school days, the textbook definition for cancer was "uncontrolled cell growth," which left me wondering: why is it harmful to the body if cell growth is high? Later I learnt that cancer cells residing in the tissue are like the weeds that grow in our garden. Like weed, the cancer cells compete with healthy cells for nutrients and hinder the growth of normal functioning cells, resulting in inhibition or decreased function of the whole organ. For this reason, most of the current cancer treatments focus on decreasing the tumor burden and paving the way to restore organ function. These cancer treatment drugs work mostly by inhibiting the DNA duplication process, thereby activating cell death. But these drugs don't particularly differentiate between normal cells and cancer cells. Even normal cells in our body undergo frequent cell division; one of the best examples is hair growth. This is the reason why most cancer patients undergoing cancer treatment suffer hair loss as a prominent side effect.

Current treatment options can only manage the disease, but not eliminate it from the body, which many times can result in recurrence of the cancer. Usually, these recurrent cancer cells are more resistant to the previously used drugs, and they need more stringent treatment options. The recurred cancer will usually present as more aggressive and spread to other organs of the body, further complicating treatment options.

I feel that the fundamental loophole in our present treatment approach is that we look at uncontrolled cell growth as the cause of the disease, but we need to understand that it is just one of the effects of cancer. What we need to target is the actual cause of the disease that is driving the uncontrolled cell growth. Here at UW-Madison, I work on two closely related but frequently

mutated genes: NRAS G12D and TP53 R172H mutations. We use lab mice as a model organism because they can closely mimic the human scenario of disease development, as they share around 97% of DNA similarity with humans. Also, most human cancer occurrences are associated with the mid-old age group (around 50 years), and mice, owing to their short life span of around 2 years, make it feasible and economical to study onset and progression of chronic diseases over their lifetime. Mice are also easy to handle when compared to other organisms like horses, pigs and rabbits. Additionally, since lab mice have been used in research for 100 years, much of mice biology is already known, which makes it easier to interpret our data.

We also use human acute myeloid leukemia (AML) and chronic myeloid monoblastic leukemia (CMML) patient samples and cell lines (which are isolated from patients with AML and then modified in the lab so that the cells will keep dividing infinitely) to validate our results that were generated in mice models. The frequency of these mutations is high in patients with AML, ranging from 15 to 27% for NRAS and 5 to 10% for TP53, and the presence of either of these mutations is closely related to a poor overall survival rate for AML patients.

I am trying to study how both these mutations work together to establish cancer and increase its aggressiveness. Using available mice and human samples, in our lab, my co-workers and I identified that AML characterized mainly by NRAS and TP53 mutations causes increased inflammation. Inflammation is a protective response by our body's immune cells towards foreign particles or cells. Inflammation is helpful to the body when it is regulated, but systemic uncontrolled inflammation can exhaust our immune cells, leading them to attack the body's own tissues, much like in auto-immune disorders.

In our research, my colleagues and I have also identified that treating mice with trametinib (an FDA approved drug to treat skin cancer) can reduce the AML cell growth. We further found that using a combination therapy of trametinib along with bortezomib (an FDA approved drug used to treat multiple myelomas) improves the survival of mice with NRAS and TP53 mutation driven AML. This is great news, because utilization of FDA approved drugs can help us reduce the time needed to translate laboratory findings to clinical use, connecting patients with better treatments sooner.

As mentioned earlier, we are trying to fight cancer mainly with drugs that can induce cell death or reduce the proliferation capacity of cells. But one major problem with cancer is that it can rapidly change its expression of proteins in such a way that it can develop resistance to previously administered drugs. The beauty of nature is the constant struggle for evolution, and it happens in cancer cells. Cancer cells strive to survive in all possible conditions, and one way they do this is by changing the expression of genes and proteins, rendering some drugs unusable. Understanding how cancer achieves this is an ongoing quest.

From my point of view, the reason our treatment approach fails is because we are trying to treat a constantly evolving, dynamic system using a drug or radiation-based approach that is static in nature. I feel the evolution of cancer cells towards resistance is more rapid and robust than the evolution of our drugs. So, we need a system that can employ an approach like evolution, and that

can also target cancer cells. This led me on my journey towards understanding the "immune system."

Immunology is the study of the immune system, which is an arm of our body's defense mechanism against foreign entities. Immune cells try to differentiate foreign particles from the body's own tissues by reading the proteins expressed on cell surfaces. Just as each person has unique fingerprints, every cell type also has a unique set of molecules that are expressed on their surface. These molecules reflect the health status of the cell. Immune cells try to create a memory of all the different particles they see by generating a subset of specialized cells called "memory cells." When the body sees the same particle again, these memory cells activate, resulting in a faster and more aggressive response compared to the first encounter. The same mechanism is used to develop vaccines - we expose our body's immune cells to molecules related to a specific virus, thereby training them to generate memory cells against that virus. When the body encounters it again the second time, we are better protected from viral attack.

I wanted to know why our bodies fail to recognize cancerous growths as foreign. Cancer cells escape the body's immune surveillance by mimicking the expression of surface proteins used by normal developing cells. Our immune cells are trained to identify the body's own cells as "self" and others as "foreign," and cancer cells exploit this trait to escape immune cells. In recent times, our definition of cancer has evolved from uncontrolled growth to "development gone wrong". Cancer cells also express proteins that can exhaust or suppress immune cell function and activity (Fig 2). Here at UW, I am trying to identify how cancer cells work to suppress immune cells. At the same time, I am searching for molecules that can boost immune system function against AML cells.

Using our mice models, I found that treatment with molibresib, which has Completed phase I clinical trials in patients with testis-related cancer, in combination with trametinib improved immune cells' ability to kill tumor cells.

Our research also aims to identify how AML cells alter the surrounding cells to support their growth. Several studies have reported that cancer cells can suppress normal cell development and enhance their own growth by secreting small molecules into the surrounding cellular environment. Specifically, I am studying how the onset of AML affects bone health. The work is still at a preliminary level, but I observed that AML cells can suppress normal bone development in mice. As I mentioned earlier in this article, most cancer patients succumb to secondary complications arising from their cancer, rather than the cancer itself. Therefore, my focus is on understanding how AML cells in the bone marrow alter a patient's heart function and contribute to heart failure.

One of the pitfalls of earlier studies was that they needed aged mice to mimic human development of heart failure in the context of AML or CHIP, as patients develop CHIP later in life, typically after 50-60 years. But the previous studies were done in young mice. Here, I am trying to use aged mice (around 20-24 months old, since the average survival of lab mice is around 2 to 2.8 years) to mimic the human condition as closely as possible.

Also, we have designed our experiments in such a way that we can shield the heart of mice (by designing lead shields that cover the chest area of mice) from other damage (in order to mimic natural development of leukemia, the aged mice are injected with AML cells by bone marrow transplantation which can sometimes damage the heart). We do this to ensure that the damage I find at the end of my study is induced by cancer, and not because of experimental procedures. The results from my study can help us develop interventions and reduce the risk of heart failure in patients with CHIP and AML. I also hope to contribute to knowledge that will help doctors cure AML entirely by employing immunotherapy. With immunotherapy, we modulate the body's immune cells by administering drugs or small proteins to boost immune cell function.

My vision is to improve the quality of life of cancer patients and the percentage of disease-free survival in them.

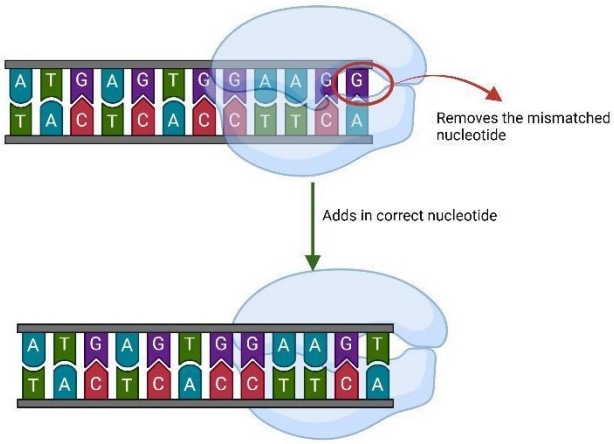

Figure 1: DNA duplication proofreading. The protein responsible for DNA duplication is called DNA polymerase and is represented as a blue structure. The alphabet represents the four different nucleotides that make up the DNA. The correct pairing of these nucleotides is A against T and G against C, if there's a mismatch of these nucleotides, the polymerase will detect and try to rectify it by replacing it with the correct one.

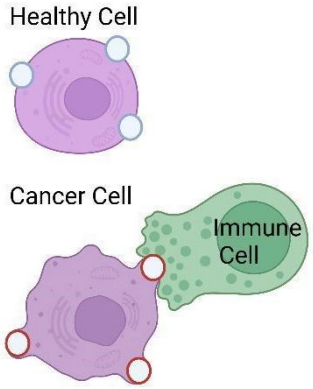

Figure 2: Representation of healthy versus cancer cell recognition by an immune cell. Red circles stand for abnormal molecules expressed by cancer cells, whereas blue circles are normal molecules expressed by healthy cells.

The above figures are created with BioRender.com