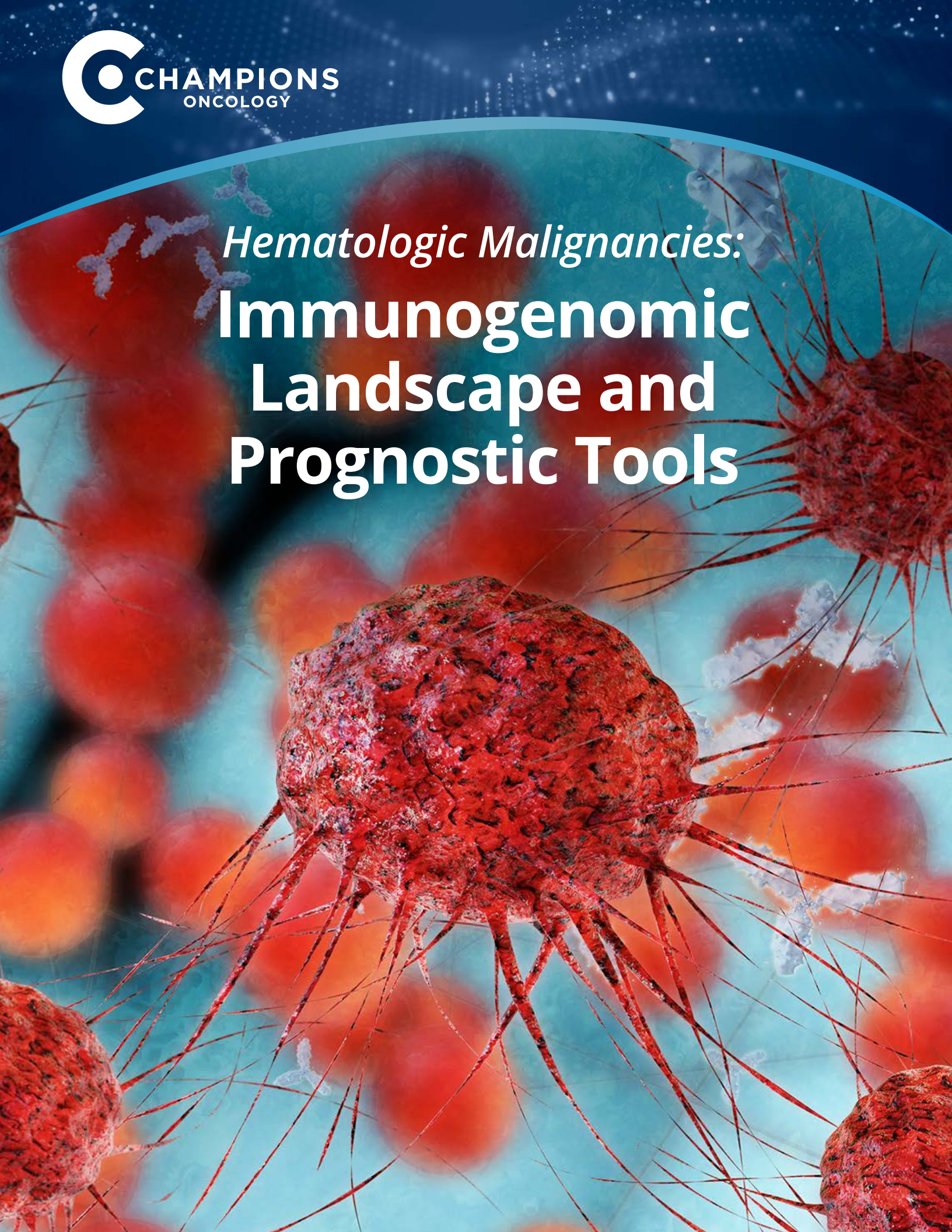
CHAMPIONS ONCOLOGY

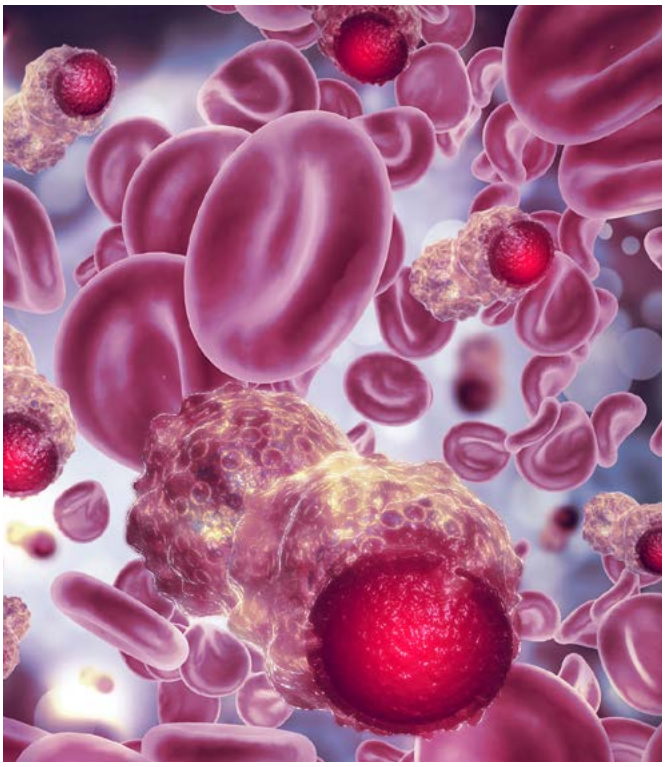
Hematologic Malignancies:

Immunogenomic Landscape and Prognostic Tools



Hematologic malignancies are blood cancers that can affect a wide range of blood cells. Malignant cells usually begin proliferating in the bone marrow, and this expansion of abnormal blood cells can lead to significant alterations in the function of the blood and immune system. Hematologic malignancies are typically categorized by cell type and generally classified as leukemias, lymphomas, and myelomas. Here, we provide overview of several of the most hematologic malignancies and highlight cutting edge technologies being used to understand these blood cancers and applying these insights toward the development of novel therapeutics and diagnostics.

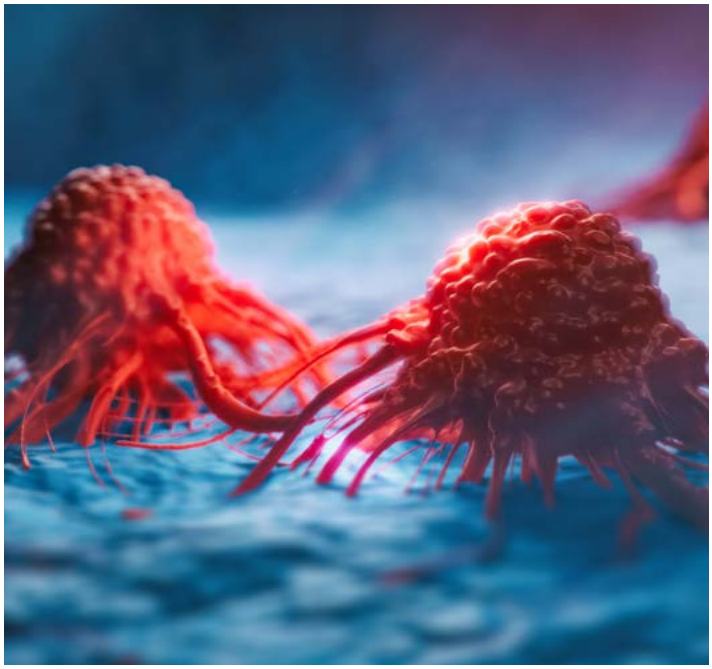
An Alphabet Soup of Blood Cancers



Most hematologic malignancies are known by their acronyms: AML, ALL, CLL, and NHL, among others. Taking note of their fully defined names gives insight into the source of each of these cancers. Most blood cancers can be classified as either acute or chronic. Acute malignancies worsen rapidly and are usually treated with aggressive therapies. Chronic malignancies can be associated with a gradual change in production of too many or too few cells and may go undetected for many years given the slow progression of such a disease.

The classification of leukemias, lymphomas, and myelomas provides insight into the cell type altered by malignancy. Leukemias typically involve cells from the bone marrow or lymphatic system, usually white blood cells. Lymphocytic leukemias affect a subset of white blood cell precursors, such as T cell blasts or B cell blasts. Myelogenous leukemias affect myeloid cells, such as myeloblasts. Malignancies that target plasma cells, which are antibody-producing B cells, are called multiple myeloma if multiple tumors are present or plasmacytoma of a single tumor is present. Features of some of the most common hematologic malignancies are outlined below:

Acute Myelogenous Leukemia (AML): In adults, acute AML is a form of cancer in which the bone marrow produces abnormal myeloblasts, red blood cells or platelets. It is the most common form of acute leukemia in adults and has been linked to smoking, previous chemotherapy, or radiation exposure. AML subtypes are classified based on the maturity of the cancer cell upon diagnosis and how it differs from normal blood cells. Childhood AML is typically caused by spontaneous mutations or chromosomal abnormalities in myeloblasts and is responsible for about 20% of childhood leukemias. In both adult and childhood AML, disease onset is associated with flu-like symptoms, bone pain, and excessive bleeding or bruising as the myeloblasts crowd out other essential immune and blood cell precursors in the bone marrow.



Acute Lymphocytic/Lymphoblastic Leukemia (ALL): Acute ALL is a malignancy of lymphocyte precursors in the bone marrow called lymphoblasts. Lymphoblasts normally develop into T cells and B cells, which are key players in the immune system. Rapid proliferation of malignant lymphoblasts are associated with flu-like symptoms, weakened immunity, bone pain and excessive bleeding as lymphoblasts expand and disrupt normal bone marrow function. ALL is the most common form of childhood leukemia and is associated with spontaneous or inherited genetic changes in lymphoblasts. ALL can also occur in adults and has been linked to previous cancer treatment and radiation exposure.

Chronic Lymphocytic Leukemia (CLL): This chronic form of leukemia typically causes expansion of lymphoblasts in older adults, although normal lymphoblasts and lymphocytes also persist. CLL is thought to be caused by spontaneous mutations in lymphoblasts, but the causes of such mutations are not well understood and have been linked to age, race, family history of blood cancers and chemical exposure. Individuals with CLL may not show symptoms at early stages of disease but typically develop enlarged lymph nodes and spleen as well as flu-like symptoms, fatigue, and greater susceptibility to infection.

Non-Hodgkin's Lymphoma (NHL): NHL is a type of cancer that occurs in the lymphatic system and causes malignancies in lymphocytes (B cells or T cells). Several different subtypes exist, and most arise from B cells (diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), and Burkitt's lymphoma). NHL can also originate in T cells, but these are less common and include cutaneous T cell lymphoma and peripheral T cell lymphoma. NHL can occur spontaneously but has also been linked to long term immunosuppressive drug treatment (in transplant recipients), certain infections, including HIV and Epstein-Barr virus, chemical exposure, and advanced age. Symptoms associated with NHL include enlarged lymph nodes, abdominal pain, flu-like symptoms, and fatigue.

Technology Toolkit

Major breakthroughs in high-throughput sequencing, epigenomics and proteomics technology, and data analysis have provided researchers with unprecedented insights into the changes in the genome, transcriptome (RNA-seq) and proteome that are associated with the progression of hematologic malignancies. One of the advantages of whole genome next-generation sequencing (NGS) and RNA-seq is that the genomes of tumor cells and normal cells from a single patient can be compared. Here we describe several of the key technologies that are being used to understand how blood cancers develop and can be applied toward identifying new therapeutic targets:

Next Generation Sequencing (NGS): This type of sequencing technology emerged in 2005 as a faster and relatively inexpensive high throughput method for sequencing DNA and RNA. Now several NGS methods exist and are based on simultaneously sequencing DNA or RNA fragments at single nucleotide resolution, which makes it a useful approach for identifying mutations or gene variants in tumor cells. All RNA sequencing methods require an addition step in which RNA is converted into cDNA by reverse transcription. No prior knowledge of the sequence is required, and this method only needs small quantities of input DNA or RNA. RNA-seq is a technique that uses NGS methods to analyze the transcriptome, or the entire mRNA transcript content of a cell or tissue, and this method is a powerful tool for quantitative comparisons between normal and tumor tissue. Another variant of NGS is whole exome sequencing (WES), which is a technique that sequences all the protein coding regions, or exons, in a genome. Sequencing of this exome requires that exonic DNA is selected or enriched prior to sequencing. WES has been especially powerful in identifying mutations in critical driver genes that are involved in blood cancer progression.

Epigenomics: This type of analysis examines epigenetic modifications on DNA or DNA-binding proteins that can alter gene expression. Epigenetic modifications are reversible and include DNA methylation or histone modifications and are critical to regulating gene expression. Tumor cells may have changes in epigenetic modifications near key genes that drive tumorigenesis, and epigenomic analysis has been a powerful method for gaining insights into these cellular changes.

Proteomics: Proteomic analysis refers to methods that measure the protein content of cells, which provides insight into gene expression within cells. Mass spectrometry is one of the commonly used tools for proteomic analysis since it can measure the mass-to-charge ratio of multiple molecules, include protein fragments. Other methods have also emerged that can better handle large proteomic studies, including one- and two-dimensional polyacrylamide gel electrophoresis, shotgun proteomics and protein microarrays. Proteomic analysis has been an essential tool for biomarker discovery in tumor cells, which has led to mechanistic insights into tumor progression and identified potential therapeutic targets.

Blood Cancers and Big Data

The first blood cancer genome that was sequenced came from an AML patient[1], and the advent of NGS technology has enabled thousands of cancer genomes to be sequenced. Now we have valuable insights into the genetic diversity of AML, which has revealed critical driver mutations that allow for the classification of different subgroups of this leukemia[2]. These analyses have revealed a role for mutations in chromatin and RNA splicing regulators and tumor suppressors.

Genomic and RNA-seq analysis has also been critical to classification of ALL subtypes that are defined by changes in ploidy (chromosome number), chromosomal rearrangements, deletions, or gene fusions[3]. Analysis of large pediatric and adult ALL cohorts has been critical to identifying genotypes associated with different responses to existing therapies[4,5], or pinpointing other therapeutic targets, such as tyrosine kinases[6]. Sequence analysis of CLL samples has revealed that this cancer has a relatively low mutation rate compared with other cancers. One unique aspect of CLL is that this cancer can be subcategorized based on analysis of whether tumor-associated B cells have undergone somatic hypermutation in the germinal centers, termed *IGHV*-mutated, or not, termed *IGHV*-unmutated[7]. The *IGHV*-unmutated subtype is associated with more aggressive disease and is more resistant to treatment[8]. Other genetic changes, including inactivating mutations in tumor suppressors or overexpression of signaling molecules, have also been shown to contribute to CLL progression[9,10].

Genomic analysis of NHL subtypes has revealed the wide range of mutations and gene alterations that drive pathogenesis. MCL is a relatively rare form of NHL but has a high relapse rate and poor survival. Recent sequencing analysis identified several new gene drivers of disease that are associated with the cell cycle, gene expression and cell death[11]. DLBCL is another aggressive form of NHL that can be refractory to treatment, and WES and transcriptomic analysis has identified missense mutations in the tumor suppressor TP53, as well as a wide range of copy number deletions and gene fusions in genes involved in cellular metabolism[12].

Biomarker Discovery and Immunotherapy Development

Genomic and proteomic studies of hematologic malignancies have not only advanced our understanding of how these diseases develop and progress but have also provided critical data for the development of targeted treatments. CLL is a disease that affects B cells and can now be treated using monoclonal antibodies that are specific to surface markers that are highly expressed on leukemic cells[13]. Rituximab is an antibody specific to CD20 that is used for targeted depletion of CLL cells and is considered a first-line treatment for patients with *IGHV*-mutated CLL. Other clinical trials have investigated the efficacy of B cell receptor inhibitors or anti-CD37 antibodies. Treatment-refractory CLL is also treated with chimeric antigen receptor (CAR) T cells, which are T cells derived from patients and engineered to express a receptor that targets CD19 on CLL cells and destroys them. Rituximab's success story for treating CLL has led to use in other blood cancers, and it is now considered a treatment option for ALL and MCL[14,15]. Given the aggressive nature, high relapse rate, and wide range of phenotypes of DLBCL, CAR T cell checkpoint blockade therapies are actively being studied as alternatives to chemotherapy[16].

Biomarker discovery has also been critical to the development of immunotherapies for AML and these studies have provided critical treatment alternatives to patients that had relapses after hematopoietic stem cell transplantation. Antibody-drug conjugates, CAR T cells and checkpoint inhibitors are all at different stages of exploration and therapeutic development for AML[17].

Collaborative Data Tools and Databases

The expansion of genomic and proteomic studies has led to the development of bioinformatic tools and databases that facilitate data mining and validation. The Cancer Genomics Hub is an example of these efforts and it functions as a repository for cancer genomic information which includes The Cancer Genome Atlas (TCGA), the Cancer Cell Line Encyclopedia (CCLE), and the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) project[18]. Researchers have also developed web servers that host large data sets and can be used for cancer prognosis and survival analysis studies based on gene expression analysis and clinical data[19]. Champions Oncology has developed its own bioinformatics software platform called Lumin, to interrogate cancer datasets from their internal model research as well as from the publicly available datasets. These tools offer researchers in silico methods to evaluate potential biomarkers for their prognostic value or identify novel immunotherapeutic targets across dozens of cancer types.

Blood Cancer Breakthroughs on the Horizon

Advances in genomics and proteomics and Immuno-Oncology research have revolutionized our understanding of hematologic malignancies and have offered patients hope for even some of the most aggressive forms of cancer. Continued research is needed to improve the efficacy of immune checkpoint inhibitors, cell signaling inhibitors, CAR T cell therapy and therapeutic antibodies. Researchers are also working to identify combinations of these therapies that have greater efficacy than individual treatments, and these combined therapies are now leading the way toward better outcomes for patients.

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