

ACUTE MYELOID LEUKEMIA, IMMUNOTHERAPIES AND FUTURE DIRECTIONS Mukati, A., & Pearson, AN.

Introduction:

Cancer, cells that mutate and form small tissues against the body's needs, is difficult to treat. When cells, such as tissue cells, undergo stresses such as prolonged sun exposure or certain chemicals, their genetic information can mutate and lead to mutations that characterize cancer, such as cancerous cells can multiply relatively fast, do not go through normal apoptosis (controlled cell death), and are unable to fix their mutations. These mutations allow cancer to become a significant burden through natural selection, as one missed cell can form a newer, more difficult tumor to treat, as only the cancer cells with the ability to evade the immune system get left behind (Dettmer, 2021).

The immune system is a group of cells that includes defense, a stored memory of the disease, and communication regulation via cytokines during infection. The immune system is the organism's primary defense against disease and sickness, including cancer. The immune system has two main branches: innate immunity, which responds quickly to infections, and adaptive immunity, which targets specific threats and can stimulate immune response and memory of infections. Together, these branches use various types of cells to identify, label, attack, and remember invaders to maintain homeostasis (Dettmer, 2021). When presented with cancer, the immune system should lead T cells and Natural Killer cells toward the tumor and enable cytotoxicity, which is the ability to kill other cells. The T cells have receptors that can detect tumor proteins presented on the cancer cells through Major Histo-Compatibility class I (MHC I) antigen-presenting proteins outside cancer cells (Dettmer, 2021). If a malignancy grows and advances far enough, though, it could prohibit the manufacture of MHC I proteins, leading to reduced cytotoxicity of the T cells. Another way cancer can evade the immune system is by stimulating the inhibitory receptors on T cells. Cancer can use proteins such as PD-L1 that bind to the inhibitory proteins and shut the T cells off, exploiting the metabolic pathway and reducing T cell cytotoxicity.

Immunotherapy is where immune cells or proteins such as antibodies are modified towards a researcher's needs. An example of notable immunotherapy is CAR-T cell therapy, where inactive virus proteins are repurposed to introduce genetic material into a T cell. The T cell then can produce chimeric antigen receptors that bind to specific AML antigens in the patient's body to improve anti-tumor efficacy. Immunotherapy has become an attractive option for cancer patients because it targets cancerous cells efficiently. However, there are still chances that immunotherapy could fail, such as if the cancer cell has antigens similar to self-antigens or the antigen receptors on T cells could not keep up with mutations in the cancer cells. In some clinical trials, immunotherapy has succeeded, with some patients achieving complete remission. Immunotherapy has the potential to provide long-term benefits to patients by enabling the formation of immune memory and attack cancer cells upon reoccurrence. As a result, there is a growing interest in exploring immunotherapy as a viable cancer treatment option.

Disease Overview:

AML has been clinically characterized by the over-accumulation and expansion of immature myeloid cells that grow through the bone marrow and peripheral blood (Vago, 2020). AML, like other cancers, is caused by abnormal mutations in cellular DNA. Specifically, the cells that make up bone marrow have DNA mutations that lead to abnormal blood and leukocyte cell formation. The bone marrow produces abnormal myeloblastic leukocytes that can crowd out normal, healthy cells in the peripheral blood and bone marrow. Chemotherapy drugs, such as mechlorethamine, procarbazine, and chlorambucil, can have some unexpected side effects on the formation of myeloblasts and can substantially impact the strength of mutations.

Mutations leading to AML would be FLT3, KRAS, NRAS, PTPN11, NF1, and KIT, which appear through signaling and kinase pathway molecules around two-thirds of the time (Vago, 2020). These

mutations, caused by genes *DNMT3A*, *TET2*, *ASXL1*, *IDH1*, and *IDH2*, are involved in epigenetic regulation and are responsible for most cases of mutation-driven Age-Related Clonal Hematopoiesis (ARCH). ARCH can be standard for individuals over 70 whose DNA mutations lead to it. Those patients who have developed *de novo* AMLs directly have mutations *RUNX1*, *CEBPA*, *FLT3* (one mentioned above), or *MLL* (mixed lineage leukemia) but not mutations associated with the group of cancers of Myelodysplastic Syndromes (MDS).

Liquid cancers, in general, do not have a particular life expectancy. Still, the National Cancer Institute states a 29.5% chance for a five-year survival rate. However, the average age of people diagnosed with AML is 65; The American Cancer Society shows a 65-70% overall five-year survival rate for pediatric AML.

For AML, the therapies have been changing massively over years of research, but improvements in immunotherapy for AML have skyrocketed. Immunotherapy has become one of the main treatments for AML and the most heavily studied. As mentioned previously, the drugs often associated with chemotherapy treatments have been flagged chronically as one of the good causes of the mutations that manifest the disease.

Immunotherapies:

For Acute Myeloid Leukemia (AML), the leukemic cells are often stimulated by the downregulation of the proteins that regulate T cells. Leukocyte Immunoglobulin-Like Receptor B4 (LILRB-4) is an immune inhibitory receptor for T cells. It is present in immune and monocytic AML cells (Mi Deng, 2018). When researchers had LILRB-4 as a knockout, the T cell suppressive ability of AML was reduced significantly (LILRB-4-KO). However, the ability of AML to decrease cytotoxicity increased when the wild-type of LILRB-4 (LILRB-4-KO-WT) was reintroduced as a test. Blocking LILRB4 increased cytotoxicity in T cells and cytokine release by them (Mi Deng, 2018). These tests have suggested that AML has been exploiting the intracellular pathways of T cells. AML immunotherapy has many strategies that promise different modifications from antibodies to T cells. However, a recent study promised a new way to treat AML (Bakhtiyaridovvombaygi, 2023). Natural killer cell (NK cell) based therapies have been undervalued because of NK cells' short lives and their hard-to-culture nature with non-specific responses. These shortcomings changed when the discovery of Cytokine-Induced Memory-Like NK Cells (CIML NK Cells) led to increased cytotoxicity. These cells achieved a long-term lifespan by pre-activating the then-normal NK cells with the combination treatment of cytokines IL-12, IL-15, and IL18. The cytokines shift the NK cell from short-term cytotoxicity to long-lived memory and antigen-driven clonal expansion (Mujal, 2021). In addition to this immunotherapy, past trials have deemed CIML-NK Cells safe to use and can generate positive outcomes for the patient, supporting the efficacy of NK immunotherapy (Bakhtiyaridovvombaygi, 2023).

A promising and commonly used immune cell for immunotherapy is CAR-T Cells. Promising targets for testing CAR T cells are CD33 and CD123 and the folate receptor β , CLL1, FLT3, LeY, NKG2D ligands, CD70, and CD44v6 (Vago, 2020). Another alternative approach is genetically modifying T cells with a high-affinity TCR (T cell receptor) specific to the AML antigen. Despite the limitations imposed by the MHC haplotype HLA and the efficiency of processing and presenting the therapy, the strategy could still be pursued by utilizing a more generalized set of antigens that would not require T cells to be specific to a single antigen (Vago, 2020).

Innovation and future directions:

AML has many different clinical studies that are researching immunotherapy. One clinical trial showcases a popular therapy, titled "Donor-derived CAR-T Cells in the Treatment of AML Patients" (NCT04766840). The clinical trial studies donor-derived CAR-T cells to treat relapsed or refractory AML.

The study involves nine patients and nine donors, and the CAR-T cells will be infused intravenously into the patients in a dose-escalating 3+3 design, where, over time, the dose gets increased by three partitions at a time. The goal is to evaluate the safety and efficacy of this treatment in AML patients who have not responded to other treatments. If successful, this could lead to new therapies for AML patients using the CAR-T cells.

As for the inclusion criteria, the study needs patients with refractory or relapsed AML. For a more accurate and safe trial, the patients must have adequate liver, kidney, heart, and lung function and be over 18 years old. The enrolling patients must also have an ECOG survival and disease progression score of 0 - 2 points, demonstrating the patient's health and functioning of daily activity (NCT04766840). However, the trial excludes patients with confirmed acute promyelocytic leukemia, recent symptomatic central nervous system leukemia, and autoimmune disorders such as graft-versus-host disease. To avoid interfering with the drug trials, the patient should not have used gene therapy products or participated in other clinical trials in the last 30 days (NCT04766840).

Scientists studied the safety of donor-derived IM73 CAR-T cells as a treatment for relapsed or refractory AML. To prepare the patient's body for the cell infusion procedure, they will receive three days of Fludarabine and three days of Cyclophosphamide two days prior. Then, they add the IM73 CAR-T Cells Drug: a modified version of T cells in which researchers attach chimeric antigen receptors that guide the T cells towards cytotoxicity-induced stages in the presence Leukemia-specific Antigens (LSA's) (NCT04766840). As mentioned before, CAR-T cells could work with a generic set of AML antigens. The CAR-T cells have an extensive repertoire of those antigens they could target. However, one approved by the FDA is CD19, a B cell maturation agent that gets presented of B cell leukemias.. Adding chimeric antigen receptors increases the cytotoxicity and limits the cancer's ability to evade immune response. The study also measured dose-limiting toxicity as an adverse event related to CAR-T cell infusion in Grades 4 or higher as the primary outcome measure. As for the secondary measure, they analyzed the objective response rate, which measured patients who achieved CR (complete response) or CRi 28 days after the CAR-T cells infusion (NCT04766840).

Conclusion:

AML, Acute myeloid leukemia, is a form of liquid cancer that belongs to the myeloid classification. The immature myeloid cells expand from the bone marrow into the peripheral blood, evading anti-tumor immune responses such as T cell cytotoxicity. AML has had many breakthroughs in immunotherapy, and it has become increasingly treatable over the past decade. Many scientists have developed immunotherapies that have focused on immune cell modifications. CAR-T cell therapy has been a modification that led to amassed cytotoxicity and is a theory in which researchers are interested in. Many researchers are looking for ways to boost the immune response when in the presence of AML antigens. Modifying specific cells to increase cytotoxicity is one of the main goals, and multiple efforts have proven successful. CAR-T and CIML-NK cell development is a promising approach to eliminating AML-suppressing immune responses by clinical T cell inhibition receptors. All studies introduced in this research have demonstrated a commonality in their potential for future success. Immunotherapy has the potential to simplify the treatment process of AML. Effortless to implement with low side effects, such as side mutations. Among various therapies, immunotherapy has shown remarkable potential for patients and researchers. Immunotherapy could address the evasion fraction of the cancer problem and benefit humanity with multiple ways.

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