Use of Immunotherapy in B-Cell Acute Lymphoblastic Leukemia

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Overview of Cancer:

Cancer is a disease characterized by the unregulated cell growth of abnormal cells which possess the capability to invade and damage healthy body parts around existing healthy tissue. The immune system is the defense system of the body composed of specialized organs and cells working cohesively to identify and destroy foreign pathogens, infections, and malignancies. Typically, the immune system recognizes and destroys malignant cells; however, cancer can circumvent immune intervention by downregulating antigen presentation (MHCs), altering tumor environments, as well as secreting immunosuppressive factors. Immunotherapy is a type of treatment designed to redirect and amplify immune response to target and kill cancerous cells. Immunotherapy is a particularly attractive cancer treatment because of its capability to leverage the immune system to destroy malignant cells without damaging healthy tissue in the process. Unlike treatments such as chemotherapy and radiation, immunotherapy can be utilized to recognize tumor antigens and adapt to changes in tumors. This can induce immune memory, allowing fightback against relapse and treatment resistance. In this review, we will discuss the methods in which B-cell acute lymphoblastic leukemia (B-ALL) evades the immune system, discuss contemporary immunotherapies and their mechanisms, and discuss the future direction of immunotherapy in B-ALL treatment.

Overview of Acute Lymphoblastic Leukemia:

Acute Lymphoblastic Leukemia is a very aggressive hematologic malignancy involving the overproduction of overly immature lymphoid cells, primarily in the peripheral blood and bone marrow. This uncontrolled growth of immature lymphoid known as lymphoblasts disrupts regular blood cell production, leading to a significant shortage of functional blood cells (Pui & Evans, 2006). ALL is the most common cancer among all children ranging in ages between 2 and 5, but is also frequent in adult males (Hunger and Mullighan, 2015). ALL is a disease that does not have many regional discrepancies, rather affecting a broad group of demographics all throughout the world. The overall etiology of Acute Lymphoblastic Leukemia (ALL) is linked to a mix of genetic predispositions, mutations, and environmental factors. Genetic mutations play the most crucial role in its development. B-Cell Acute Lymphoblastic Leukemia(B-ALL) is a subtype of ALL, in which immature B lymphocytes expand uncontrollably in the bone marrow. One of the most significant mutations is the BCR-ABL1 gene, which is formed by a translocation between chromosomes 9 and 22, creating what is known as the Philadelphia chromosome (Pui & Evans, 2006). This mutation is strongly associated with a poor prognosis, as it leads to the uncontrolled growth of abnormal white blood cells. This mutation causes the creation of a protein called tyrosine kinase which acts as a cell accelerator that causes white blood cells to be produced uncontrollably (Pui & Evans, 2006). Other genetic mutations that result in ALL are mutations involving the MLL gene which causes parts of the MLL gene to break off and fuse with other genes(MLL fusion genes), producing proteins that cause the activation of genes responsible for uncontrolled cell growth. Similarly, mutations in the NOTCH1 are responsible for many cases of T-Cell ALL, in which the NOTCH1 receptor sends continuous signals to T-Cells to grow uncontrollably, contributing heavily to the development of leukemia (Pui & Evans, 2006). ALL is a disease that affects a multitude of demographics, while showing an evident spike in children.

Additionally, men are more likely to be diagnosed with ALL compared to women, with cases also increasing directly with age for adults. The disparity with gender related diagnosis is yet to be identified, while certain genetic conditions cause an increased chance of ALL, such as individuals with Down Syndrome (Hunger and Mullighan, 2015). Pediatric patients often experience higher chances of survival, with survival rates often exceeding 80%. However, adults face a significantly lower survival rate especially as age increases. Patients with ALL are often treated in an approach that incorporates many phases, often starting with chemotherapy that looks to kill leukemia cells, followed by differing types of therapy. For patients that have the Philadelphia chromosome, targeted therapies such as tyrosine kinase inhibitors (TKIs) are used to suppress excessive cell signals to inhibit cancer cell growth. Immunotherapy is still developing for ALL, and has shown some promise. CAR-T cell therapy is an immunotherapy that engineers a patient's T cells to express receptors known as CD19, a protein found in leukemia cells. Stem cell transplants as well as radiation therapies are also increasing in prevalence, but are still relatively less common.

Use of Immunotherapy in B-ALL:

B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive hematologic malignancy arising from the abnormal production of immature B cells. Many methods of immune evasion and multiple immune suppression mechanisms that contribute to anti-leukemic response exist, one key mechanism being the production of transforming growth factor beta (TGF-β) from leukemia cells, which initiates the SMAD cascade in natural killer cells. This initiation suppresses NK cell cytotoxicity by impairing granzyme and perforin expression, allowing for the proliferation of leukemia cells despite immune system interaction (Rouce, Shaim, Sekine, Weber, Ballard, Ku, Barese, Murali, Wu, Liu, Shpall, Bollard, Rabin, and Rezvani, 2016). Additionally, leukemia blasts can control cytokine secretion and can repress MHC genes which leads to weaker T cell activation and slower immune detection (Aureli 2023). The dysregulation of NK and T cells allows for the spread of malignant cells while evading immune detection. Many immunotherapy strategies have been developed for B-ALL treatment, involving chimeric antigen receptor (CAR) T cell therapy, bispecific T cell engagers (BiTEs), antibody drug conjugates (ADCs), and BCL-2 inhibitors. CAR-T cell therapy, specifically targeting CD19, involves the genetic modification of autologous T cells to express receptors that target CD19 on B-ALL cells, initiating cytotoxicity without MHC expression. Tisagenlecleucel is the CD19 targeted CAR T cell therapy product, which was approved by the FDA for pediatric and young adults with relapsed and refractory B-ALL (Aureli 2023). This has demonstrated complete remission rates of up to 81%. Another immunotherapeutic approach is the use of blinatumomab, a BiTE antibody treatment that links CD3, a T cell co-receptor, on T cells to CD19 on B-ALL cells. This antibody reinstates T cell production and cytotoxicity and is FDA approved for the treatment of minimal residual disease positive and relapsed B-ALL (Aureli 2023). Ongoing clinical trials have displayed remission rates higher than 75%, with higher survival rates compared to chemotherapy. An example of a commonly used ADC is Inotuzumab ozogamicin, which targets CD22 and delivers a cytotoxic agent directly to leukemic cells. This treatment is FDA approved, and has shown some efficiency in adult patients with relapsed B-ALL. New targeted immunotherapies have increased in prevalence outside of previously used approaches. Venetoclax is a BCL-2 inhibitor that has become a promising approach in preclinical models addressing KMT2A rearranged B-ALL, a type of ALL that has a very poor prognosis. The conduction of *in vitro* and *in vivo* testing have

shown that venetoclax induces apoptosis in KMT2A rearranged B-ALL cells, showcasing its potential as an alternative or continuation of current immunotherapies (Richter, Lange, Holz, Brock, Freitag, Sekora, Knuebel, Krohn, Schwarz, Hinz, Escobar, and Junghanss, 2022). Despite constant improvements, there are many limitations still present. CAR T cell therapy can lead to life threatening disorders such as cytokine release syndrome (CRS), an inflammatory reaction, and immune effector cell associated neurotoxicity syndrome, a neuropsychiatric condition (Aureli, Marziani, Venditti, Sconocchia, and G Sconocchia, 2023). These syndromes require significant amounts of management, creating life threatening issues for patients using immunotherapy. Similarly, the efficiency of drugs like blinatumomab and Inotuzumab is prone to reduction due to differing expression levels of their antigens and treatment toxicities.

Innovation and Future Directions:

B-cell acute lymphoblastic leukemia (B-ALL) is a high-risk hematologic malignancy that avoids immune response through downregulation of major histocompatibility complex (MHC) molecules and secretion of immune-suppressive cytokines, for example, transforming growth factor-beta (TGF-β) (Rouce, Shaim, Sekine, Weber, Ballard, Ku, Barese, Murali, Wu, Liu, Shpall, Bollard, Rabin, and Rezvani, 2016). With a dependence on immunotherapy as part of its advanced treatment, many clinical trials are underway to discover new therapeutic approaches to treatment resistance and subsequent relapse. The clinical trial is a Phase I trial using autologous T-cells with a chimeric antigen receptor (CAR) directed towards CD19 to determine safety and efficacy in a combined pediatric/adult population diagnosed with relapsed/refractory CD19-positive B-ALL (1-26 years old) (NCT01860937). Inclusion criteria consist of receiving previous therapy and standard-of-care relapsed/refractory treatment with a high expression of CD19 on leukemic cells; exclusion criteria are active central nervous system disease, active infections, and patients who underwent previous gene therapy. The intervention is CAR T-cell transduction via a lentiviral vector; primary endpoints include safety, feasibility, and engraftment. This trial is significant in expanding CAR T-cell therapy population to younger patients while assessing engraftment longevity and risk for relapse in patients with more aggressive disease. Furthermore, the trial addresses shortcomings of prior studies such as antigen escape due to the prolonged monitoring for CD19 expression and tolerability of patients over time. Another significant ongoing trial is NCT05460533, a Phase I study testing dual-targeting CAR T cells that simultaneously recognize both CD19 and CD22, two antigens commonly expressed on B-ALL cells. This study enrolls adult patients with relapsed or refractory B-ALL. This study enrolls especially those patients who have failed several previous therapies, including several CD19-targeted treatments. The dual-target approach has the aim of helping to reduce the problem of antigen loss—a quite common cause of relapse after CD19-specific CAR T cell therapy—by providing redundancy in targeting, making it more difficult for leukemic cells to then escape immune recognition. Exclusion criteria prominently include active autoimmune disease, uncontrolled infections, as well as palpable organ dysfunction. This trial assesses safety and tolerability while monitoring antileukemic activity of the dual CAR T cell contributing to the further development of next-generation CAR technologies (NCT05460533). Future directions in B-cell acute lymphoblastic leukemia immunotherapy aim to control many challenges that limit the success of long-term success. One major focus is the development of next-generation CAR T cells that provide enhanced persistence in the patient's body, while also reducing side effects such as cytokine release syndrome and neurotoxicity (Aureli, Marziani, Venditti, Sconocchia,

and G Sconocchia, 2023). Researchers have been making modifications to pre-existing CAR cells by altering co-stimulating receptors and further regulating cytokine production, primarily to minimize adverse effects such as neurological toxicity (Aureli, Marziani, Venditti, Sconocchia, and G Sconocchia, 2023). Additional emerging techniques include the use of combination therapies which utilize immunotherapy with conventional tactics, such as chemotherapy. The combination of these approaches aim to target leukemic cells through many simultaneous methods, enhancing effectiveness and reducing chances of treatment resistance. Currently, the pairing of apoptosis modulators such as venetoclax, a type of BCL-2 inhibitor, with CAR T cell therapy has shown signs of interdependent potential in preclinical diagrams. This has shown significant progress in KMT2A-rearranged B-ALL, a high risk subtype of ALL (Richter, Lange, Holz, Brock, Freitag, Sekora, Knuebel, Krohn, Schwarz, Hinz, Escobar, and Junghanss, 2022).

Conclusion:

B-ALL is a subtype of ALL, in which immature B lymphocytes grow uncontrollably in the bone marrow. The use of immunotherapy in treatment for B-ALL has improved outcomes in cases with relapse and resistance because of its specific ability to target leukemic antigens and sustain immunity. Modern therapies such as CD-19 targeted CAR T cells, bispecific antibodies such as blinatumomab, and drug conjugates have increased remission rates within pediatric patients. Current research targets the development of next generation CAR T cells, finding new antigen targets aside from CD19 and CD22, and reducing harmful side effects such as neurotoxicity and cytokine release syndrome. In the future, immunotherapy has the potential to enhance long-term survival and provide the potential of curative treatments due to contemporary advancements in B-ALL care.

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