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Review

A Thorough Investigation into the Technology of Kymriah – Tisagenlecleucel A CAR T Cell Immunotherapy

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Abstract

Tisagenlecleucel, commonly known by its brand name, Kymriah, is a CAR T cell immunotherapy that aims to treat patients diagnosed with relapsed/refractory Acute Lymphoblastic Leukemia (r/r ALL) and Diffuse Large B Cell Lymphoma (DLBCL). Previously, the standard of care for these two hematological malignancies was chemotherapy; however, research has shown that only 50% of patients who receive chemotherapy go into remission.[1] Additionally, chemotherapy is an aggressive approach with severe side effects and inconsistent results. With the new development of the CAR T cell technology, there is hope for long term survival from these life-threatening cancers. Kymriah is one such therapy that uses CAR T technology to re-engineer a person's own T cells to attack cancer in the body. Two main efficacy studies were done for Kymriah JULIET and ELIANA that showed patients with a complete response had a 90% probability of survival at 12 months for DLBCL, and an 81% remission rate for ALL, respectively. Despite the incredible results, there are some limitations to this innovative CART cell therapy including debilitating side effects and the complete elimination of all B cells. Further research into this groundbreaking technology will surely allow for a true treatment for a debilitating disease.

Introduction

Kymriah is the brand name of the drug Tisagenlecleucel, produced by the biotechnology company Novartis. It is a CAR T cell immunotherapy that aims to treat patients diagnosed with two major cancers; relapsed/refractory Acute Lymphoblastic Leukemia (r/r ALL) and Diffuse Large B Cell Lymphoma (DLBCL). ALL is the most common childhood cancer with a prevalence of 1/1000 and an incidence of 5,960 cases annually, as of 2021.[2] ALL is an aggressive leukemia characterized by the uncontrollable proliferation and persistence of abnormal lymphoblasts or lymphocytes in the bone marrow, peripheral blood, or extramedullary

sites.[3] DLBCL is the most common type of Non-Hodgkin's Lymphoma, with 18,000 new cases reported each year.[2] It is characterized by abnormally large B-lymphocytes (B Cells) that stop responding to signals, which halt growth and division in the lymph nodes, skin, breast GI tract, brain, and bone. B cells are responsible for producing antibodies.[4]

Previously, the standard of care for these two hematological malignancies was chemotherapy. However, research has shown that only 50% of patients who receive chemotherapy to treat localized and advanced stages of DLBCL go into remission.[1] Additionally, chemotherapy is an aggressive approach with severe side effects and inconsistent results. As such, traditional methods for treating these cancers must be further explored.[5] With the new development of the CAR T cell technology, there is hope for long term survival from these life-threatening cancers. Kymriah is one such therapy that uses CAR T technology to re-engineer a person's own T cells to attack cancer in the body.

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Development of CAR T Cell Immunotherapy

The development of CAR T cell immunotherapy began with the discovery of T cells in the 1960s by Immunologist Jacques Miller.[6] In 1992, Dr. Michel Sadelain first attempted to engineer a T cell.[7] In 1993, the first generation of chimeric antigen receptors (CAR) was developed at the Weizmann Institute. Despite the CARS being successfully created, they were not ready to be used for medical purposes until proven that they could survive in the body.[8] Scientists successfully created a CAR in 2002, that could survive in the body, proliferate, and kill cancer cells by adding a co-stimulatory molecule (in this case CD28) to the re-engineered T cells.[9] In 2009 the manufacturing procedure of CAR T cells was released for use in human beings.[10] This was a major step in medicine as the FDA declared CAR T cell therapy a "breakthrough" therapy.[11] In 2017 the FDA approved Kymriah for the treatment of pediatric and adult patients with ALL, and in 2018 for DLBCL.[12] [13] Thus, the Kymriah

therapy is a result of the effort made over the span of 6 decades to find an immunotherapy that incorporates the usage of chimeric antigen receptors on T cells. In 2018, James Allison was awarded the Nobel Prize for his discoveries about the biologies of immune T cells and his invention of immune checkpoints to treat cancer.

Function of Kymriah CAR T

Kymriah CAR T cell immunotherapy functions by reprogramming a patient's T cells into CAR T cells. The Kymriah CAR allows the T cells to identify and eradicate malicious CD19 expressing cells. It is comprised of a CD19 specific, murine single-chain antibody fragment (FMC63) followed by a CD8-α hinge and transmembrane region, which is fused to both the 4-1BB costimulatory domain and the intracellular CD3-ζ signaling domain (Figure 1).[14] The 4-1BB Domain acts as the costimulatory signal required for T cell activation and is essential for the persistence, expansion, and antitumor activity of Kymriah. The technology uses a lentiviral transduction vector (LV), which can infect both nondividing and dividing cells, to deliver the transgene that encodes the CD19 specific CAR into the host cell's chromosome responsible for gene expression. The LV initiates the binding, ex vivo, of reprogrammed T cells to the CD19 receptor on target cells, which results in T cell activation as well as initiation of target cell destruction and cytokine production.[14] LV does not obtain the specificity required for in vivo applications, such as cell-specific LVs, which is currently a major drawback for the entire gene therapy field.[15] The lack of specificity can result in off-target transductions, which leads to adverse effects, such as insertion mutations causing inhibition of anti-oncogenes and activation of proto-oncogenes. Despite the potential risk factors, LVs are considered one of the safest options available, as they are associated with high levels of safety with minimal immunogenicity reported, as well

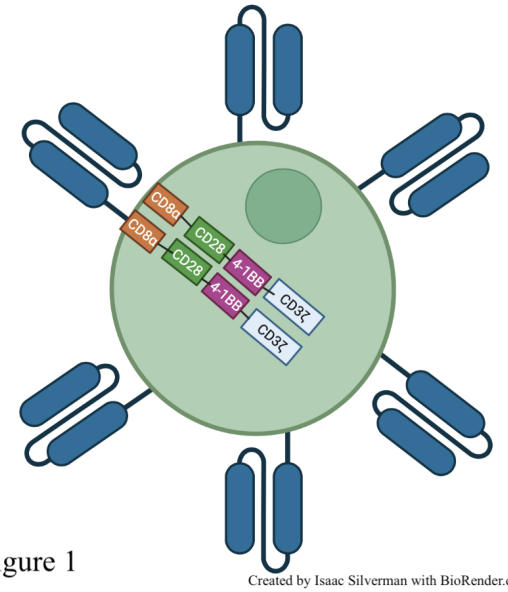

Figure 1

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as rapid induction of transgene expression. This process does not require the elimination/ disruption of any genes.[15]

DLBCL and ALL were specifically chosen as viable targets for treatment with Kymriah because they are types of B-cell malignancies that always demonstrate CD19 expression. CD19 is a cell surface protein receptor whose expression is restricted to B cells. When treating DLBCL and ALL, CD19 CAR-T cells can be utilized, as they are engineered to identify and attack the CD19 antigen on the cancerous B cells. However, Kymriah can't treat all B-cell malignancies, such as certain leukemic cells, because their CD19 expression is often masked on cancerous B cells. Since DLBCL and ALL maintain consistent CD19 expression, they were the ideal targets for treatment with Kymriah.[16]

The Kymriah CAR-T cell manufacturing process occurs in four steps and begins with the collection of non-mobilized, peripheral blood, mononuclear cells from a patient through leukapheresis. In this process, the patient's white blood cells (T cells) are extracted. All remaining cells and plasma are returned into the bloodstream. The T cells are cryopreserved within 24 hours after collection and frozen (Figure 2).[14]

The second step is cell manufacturing, in which the extracted T cells are sent to a laboratory and treated. The T cells become enriched, selected, and activated by the use of anti-CD3/CD28 antibody-coated paramagnetic beads. These beads allow for ongoing cell stimulation and are linked to higher T cell activation and cytokine production, compared to activation, another method, with anti-CD3 and interleukin (IL)-2. These T cells then get transduced

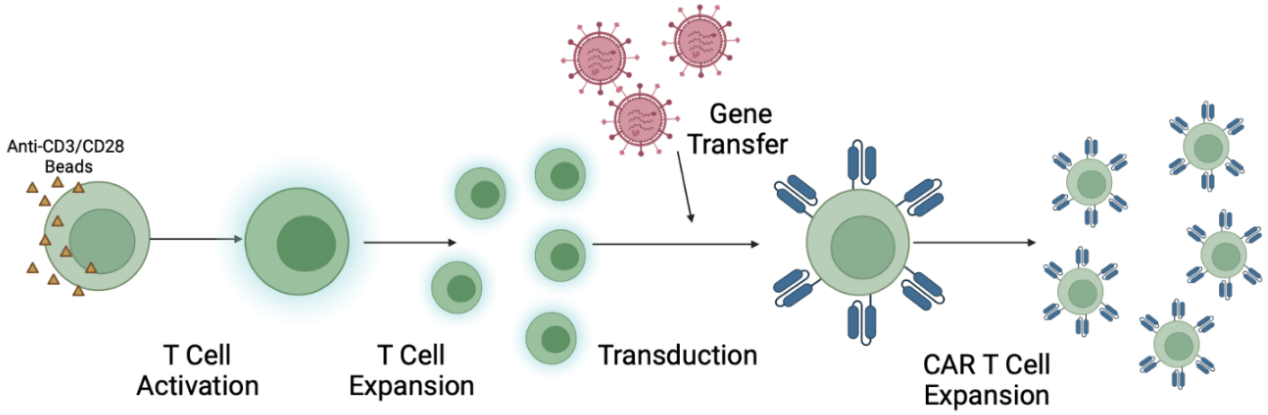

Figure 2

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with a lentiviral vector that is self-inactivating and contains anti-CD19 CAR transgene. Once transduced, the beads can select and activate T cells until expansion ends. This reduces the unwanted loss of antibody stimulating antibodies during the process of media exchange.[17] The unused vector is eliminated from the cells after transduction. The cells are then grown in culture. Cell expansion continues ex vivo until there are enough cells to meet the dosage requirement. The transduced T cells are harvested through isolation and separated from the paramagnetic beads, and then are processed into an infusible media.

In the third step, infusion, the infusible media, made of the re-engineered T cells, is transferred into an IV bag, cryopreserved, and shipped back to the medical facility. Thereafter, it is administered to the patient via one direct infusion. Steps one through three can take three to six weeks. Patient monitoring, the fourth step, consists of following the patient's reaction for extended periods of time.[14]

Efficacy

Two main efficacy studies for Kymriah are JULIET and ELIANA. Both were open-label, multicenter, single-arm, phase 2 global trials for Kymriah. The JULIET study targeted adult patients with r/r DLBCL to determine the best overall response rate. There were 115 participants, who had at least two previous lines of therapy- including rituximab and anthracycline. The overall survival rate is depicted from the date of infusion until the date of fatality, of the total patients that were given Kymriah.[18] Patients that exhibited any level of response had a 49% estimated probability of survival, while those with complete responses had a 90% probability of survival, at 12 months.

The ELIANA study targeted pediatric and young adults with r/r ALL to evaluate the overall rate of remission, which included either complete remission (CR) or CR with incomplete blood count recovery (CRi), with at least three months of follow up. The 75 patients deemed eligible, once receiving prior anti-CD19 therapy, were enrolled and monitored. Impact was documented for 12 months after study completion. Overall rate of remission reported was 81% for patients who received the infusion: 60% had CR, while 21% had Cri. Patient overall survival rate was 90% at month 6 and 76% at month 12.[19]

Limitations of Kymriah

Although Kymriah has given many patients a second chance, there are various limitations. Some obstacles surrounding the mechanism of the drug include debilitating side effects and the complete elimination of B cells. Side effects include cytokine release syndrome (CRS) and neurological toxicities. CRS is the result of overstimulation of the innate and adaptive immune system, which causes the release of cytokines that are toxic to other cells. The occurrence of CRS can lead to a cytokine storm, where the body is flooded with cytokines often resulting in severe toxicity, organ failure, and death.[20] Another limitation is that when the T cells are reprogrammed the inserted CAR remains in the genome in perpetuity, as such, B cells are constantly being attacked and destroyed. Individuals who receive Kymriah don't retain any B cells, resulting in a need to take daily medications to replace the lack of antibodies in their system.[21]

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The treatment also has some technical limitations including long vein-to-vein time, a requirement of pre-treatments of chemotherapy, high cost, and issues with scalability. Vein-to-vein refers to the time it takes from when blood is drawn to when the blood is reinfused. Oftentimes patients with late-stage cancer cannot afford to wait. Patients also may not be able to get the treatment due to the requirement of chemotherapy, making them too weak to survive. Additionally, the high cost of treatment and limited facilities capable of processing the T cells, make it challenging for many patients to receive Kymriah.[22] [23]

Advantages of Kymriah

Kymriah has multiple advantages to the standard of care on the market today. CAR T cell therapies are a type of personalized medicine in which an individual is treated with their own T cells. As such, there is no risk of rejection and no need to wait for a donor. Additionally, the fact that the treatment only requires one infusion gives reprieve to patients who have received many treatments.[24] Most importantly, Kymriah has a higher success rate relative to classic chemotherapies, such as Clofarabine. In a study done in 2020, remission rate and success rate were studied, comparing tisagenlecleucel to Clofarabine for the

treatment of ALL. The study spanned two years and followed 183 patients who were divided by their treatment plans, Clofarabine or Kymriah. After a year, the results indicated that tisagenlecleucel performed significantly better with a 45% higher survival rate when compared to Clofarabine.[1] [25] Additionally, CAR T cell therapies also provide longer remission rates compared to chemotherapies on the market. The five-year remission rate for ALL after rounds of clofarabine chemotherapy ranges between 10%-20%, CAR T cell therapies report 46% of patients remain cancer-free and in remission at the five-year follow up.[26]

Compared to another CAR T cell therapy, Yescarta, Kymriah has shown higher efficacy and has been approved for a broader range of indications. Kymriah treats adults and pediatrics, while Yescarta only treats adults for DLBCL. Both target CD19 antigens; Kymriah T cells are programmed with a lentiviral vector while Yescarta T cells are programmed using a ϒ-retroviral vector.[20] A study done in 2019 comparing the two therapies, showed that 62% of ALL patients and 64% of DLBCL patients who receive Kymriah reach remission. Only 51% of patients with DLBCL reach remission with Yescarta.[27] Kymriah has thus far been the most successful treatment in the CAR T immunotherapy arena.

Conclusion

CAR T cell therapy has made such a significant impact that further applications are already being researched. A study called PORTIA is focused on the effects of simultaneously treating patients with Kymriah and Pembrolizumab, which is a PD-1 inhibitor. PD-L1 and PD-1 are the proteins involved in cancer development. PD-1 (programmed cell death) delivers negative signals upon interaction with its ligand PDL-1. PD-L1 has a broad range of immunoregulatory roles in the cells.[28] PD-L1 masks the cell marker for cancer by attaching to PD-1, allowing cancer to develop. Pembrolizumab is a drug that inhibits the function of PD-1, prohibiting attachment to the cancerous cell, thereby eliminating it. This study included patients who unsuccessfully underwent CAR T cell therapy as an attempt to salvage it.[29] There is also ongoing research for the usage of Kymriah with other cancers, for example, r/r Follicular Lymphoma.[30]

We have reached a point where immunotherapy treatments are a main focus within biotechnology, as there is hope to

cure diseases in a more personalized and efficient manner. Kymriah has already progressed significantly over the years, but new advancements are becoming more realistic every day.

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