ACADEMIC REVIEW OPEN ACCESS

A Review on Anti-CD19 CAR T Therapy against B-Cell Malignancies and Future Implications

ARTICLE INFORMATION

Received: 19 February 2020
Accepted: 24 March 2020
Published: 31 March 2020

Senior Editor
Amama Khairzad

Reviewers and Section Editors
Pouriya Sadeghighazichaki
Mohammed Inam Khan

Layout Editor
Youssef El-Sayes

Illustrator
Roushan Tabassum
Simran Kaur

Milena Hurtarte[1] and Kasia Tywonek[2]

1. McMaster University, Honours Biochemistry, Class of 2021
2. McMaster University, Honours Integrated Science, Class of 2021

ABSTRACT

This review examines CAR T CD19 immunotherapy, a newly FDA approved targeted therapy for B-Cell Acute Lymphoblastic Leukemia treatment. This therapy utilizes modified T cells from the patient's immune system, engineered to possess an anti-CD19 receptor that can recognize the specific CD19 antigen expressed on the surface of malignant B-lymphocytes. Using this highly individualized treatment, cancer types with a high rate of metastasis or relapse can be treated by the targeted nature of this therapy. The review aims to summarize the process through which CAR T was developed, from its inception to FDA approval. The material examined is current until March 2019 and explores the mechanisms and management of CAR T cell toxicity experienced by patients undergoing treatment. Clinical trials from respective stages of development are also detailed and summarized. The viable treatment options for patients suffering from B-cell acute lymphoblastic leukemia (B-ALL) are outlined. Despite the promising remission rates of CAR T therapy, its accessibility is limited due to current cost of treatment. With advancements in technology and improved understanding of immune-based therapies, it is possible that this method can become a more viable and affordable treatment option for patients in the future.

Keywords: Immunotherapy, B-ALL, personalized medicine, CAR T therapy

INTRODUCTION

What is B-Cell Acute Lymphoblastic Leukemia?

Acute lymphoblastic leukemia (ALL) is a cancer which is caused by the transformation and proliferation of lymphoid type progenitor cells in bone marrow, blood and extramedullary sites.[1] B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive form of ALL characterized by the differentiation and proliferation of immature white blood cells, called B-cell lymphoblasts, in the bone marrow and in circulation due to chromosomal abnormalities and genetic mutations .[1] Statistical data from the American Cancer Society illustrates that ALL onset follows a bimodal distribution, affecting those under 18 and over 50 years of age.[2,3] There are many cytogenetic subtypes of ALL corresponding to a multitude of mutated or translocated genes which cause specific types of cancer.[4,5] The genetic variation decreases the effectiveness of general therapies when treating ALL. The specificity of each case of ALL is a foundational reason for the creation of immunotherapies such as CAR T that can be individualized to a patient's genetic history.[6]

Treatment Options for B-ALL

As a result of various mutations expressing ALL and the large age range affected by the disease, additional research is necessary for the development of treatment options that considers the genotype, phenotype, and risks involved for individual patients.[1] Individualized treatment approaches vary, but consistently emphasize remission-induction therapy followed by intensification therapy and continuation treatment to maintain remission.[1,7,8]

Chemotherapy is generally the first course of action for

most treatment plans. It is referred to as the remission induction phase, in which 99% of the initial leukemia cells are eradicated without compromising the normal function of all systems.[8] The most commonly used chemotherapeutics are glucocorticoids, such as prednisone or dexamethasone which inhibit cell metabolism to slow tumour growth.[8] A second category of systemic therapeutic agents include tyrosine kinase inhibitors (TKI) which inhibit protein phosphorylation, therefore protein function, in order to slow tumour growth.[8] This treatment can be administered in conjunction with various chemotherapies as combination regimes.[9,10] This approach is a generalization because treatment plans vary, depending on the patient's condition and potential side effects of the therapy, despite the cancer's aggressive phenotype.[11,12] Older patients are not able to handle aggressive chemotherapy doses, therefore a regime is created using both chemotherapy and a TKI to reduce side effects while still attempting to reach the goal of remission induction safely.[8,9]

Once remission is achieved, the next stage is to ensure the cancer is controlled and no longer proliferating at a high level. This phase is referred to as the intensification phase. This phase includes high doses of methotrexate and asparaginase therapies.[9,13] These treatments tend to be around 20-30 weeks in length and consist of one or more anti-leukemic drugs at high doses to reinforce the current remission.[14] The intensification phase can also include allogeneic cell transplants which transfer stem cells from healthy individuals to cancer patients in order to replace the patient's immune system cells and more effectively fight the malignant cells.[5] In fact, 45-70% of long term survival rates of adult ALL can be attributed to transplantation, compared to 30-40% attributed to chemotherapy.[1,15,16]

The last phase of treatment is the continuation or preventative phase that requires prolonged treatment once the patient is in remission.[1] Patients can be treated with potentially two more years of chemotherapy as a long term option.[1] Another option is a daily combination regimen of methotrexate and mercaptopurine which interfere with cell growth, in order to ensure white blood cell counts are normal and the immune system is functioning normally.[1]

PRINCIPLES OF CAR T CELL THERAPY

T cells are lymphocytic immune cells that protect the body from pathogens and malignant cells. The use of these engineered cells as a form of cancer therapy is a revolutionary scientific discovery, marking the beginning of a new era in medicine. This form of therapy utilizes programmed T cells expressing chimeric antigen receptors (CARs) on their surface to target tumour -associated antigens.[17] CARs are usually comprised of an extracellular domain consisting of an antigen binding moiety and a spacer, a transmembrane domain, and an intracellular domain that induces the signal transduction for T cell activation (Figure 1).[18] The extracellular antigen binding moiety is a single-chain fragment variable (scFV) derived from antibodies responsible for recognizing and binding to tumour-associated antigens (TAAs) expressed on the surface of malignant cells. The spacer component of the extracellular domain acts as the connection between the antigen binding moiety and the transmembrane domain. The transmembrane domain contributes to the stability of the receptor and anchors the CAR to the cell membrane.[18] The intracellular domain is mediated by co-stimulatory receptors that induce a signalling cascade to generate an apoptotic or necrotic immune response against malignant cells.[19] The components of the intracellular domain have evolved since the initial development of CARs in 1989 by Dr. Eshhar's group at the Weizmann Institute of Science, in Israel.[19] Currently, there are three generations of CARs comprised of variable co-stimulatory receptors within the intracellular domain.[20] The CAR T structure is dynamic, in that it has evolved since its discovery, and will continue to change with improved understanding through research on its use and future applications against solid tumours.

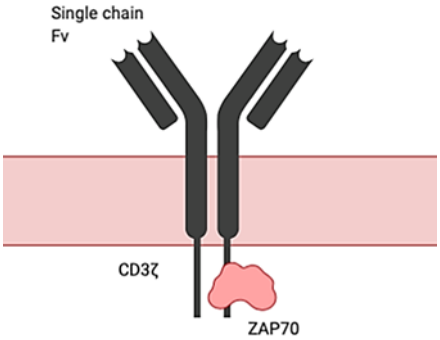

Figure 1. The model is a visual representation of the chimeric antigen receptor structure. The extracellular domain consists of the scFv from a monoclonal antibody that recognizes a TAA. The hinges and transmembrane domains are used to link the recognition domain and the intracellular signalling domain consisting of the CD3-ζ chain and co-stimulatory signalling molecules. The CAR structure is shown in complex with the signalling molecule Zap70. Image adapted on Biorender.[21]

Procedure

The inoculation of CAR T- cells in patients is a complex, multi-step process shown in Figure 2. First, T cells are collected from the patient. Autologous T cells are collected by apheresis, a procedure that withdraws blood from the body. T cells are isolated and the remaining blood components are deposited back into the body.[22,23] Then, T cells are re-engineered in a laboratory. The T cells are sent to a laboratory where they are genetically modified using non-virus and virus-

mediated transfer of nucleic acids into cells. The expression of foreign DNA produces CARs on the surface of the T cell. The CARs allow the T cells to recognize specific antigens on targeted malignant B-cells.[22,23] Next, the modified cells are grown in culture in the laboratory. This process can take a few weeks. Once a sufficient amount of CAR T cells have been expressed/grown, they are frozen and sent to the hospital where the patient is being treated.[22,23]

Prior to CAR T cell infusion, patients receive a brief course of lymphodepleting chemotherapy- referred to as conditioning therapy, to suppress the patient's immune system to prepare for incoming CAR T cell and to promote proliferation.[22,23] Next, the CAR T cells are thawed and infused into the patient's bloodstream in a process similar to a blood transfusion. These cells are then able to induce apoptotic and necrotic effects on the cancer cells that possess the targeted antigen on their surface. During this stage, patients may receive medication to prevent and/or regulate side effects (see Mitigating Side Effects section for more detail).[22,23] Lastly, patients who have received CAR T therapy have a risk/recovery period of two to three months, during which time they are closely monitored. CAR T cells may eradicate all cancer cells, however some may remain active in the body months after infusion.[22,23]

CD19 Target

One of the most investigated targets for CAR-based therapy is the CD19 antigen due to its expression in most B cell leukemias and lymphomas, but absence in cells not in the B-cell lineage.[17] The CD19 antigen ultimately triggers cell lysis, therefore targeting this antigen aims to control tumour proliferation.[17] Furthermore, anti-CD19 CAR T cells have demonstrated successful results in the treatment of relapsed/refractory (R/R) B-cell malignancies, such as Non-Hodgkin lymphoma (NHL), ALL, and Chronic Lymphocytic Leukemia (CLL).[17] Clinical trials have been conducted since the initial success of CD19 CAR T therapy and have shown complete remission in 70-94% of patients with B-cell malignancies.[17] Promising outcomes in CAR T have been shown with the targets CD19, CD20, and CD30, expressing a CD28 or 4-1BB costimulatory domain, however the most success in clinical trials has been demonstrated by targeting CD19 for B-ALL specifically.[17]

The Food and Drug Administration (FDA) and other regulatory agencies in the pharmaceutical industry have recognized CAR T therapy as a revolutionary treatment and have approved two anti-CD19 CAR T

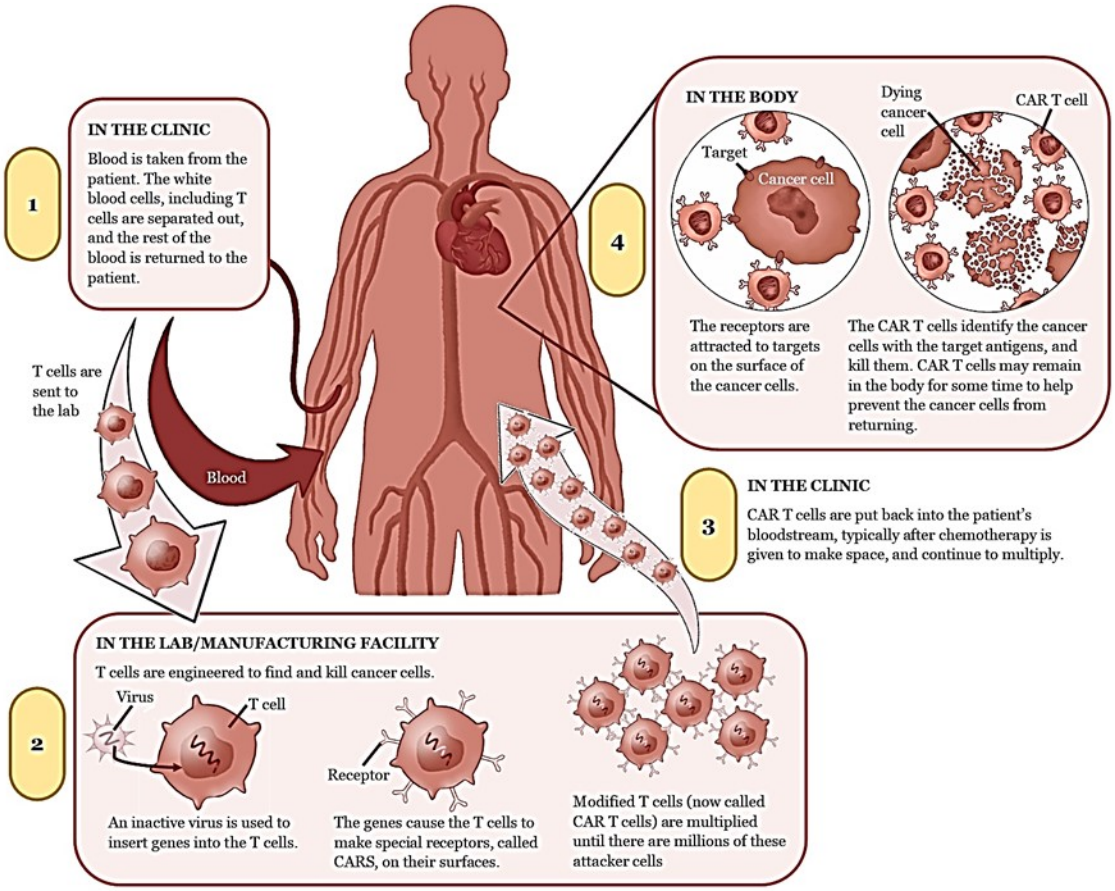

Figure 2. Visual representation of the multi-step CAR T cell immunotherapy procedure for infusing the genetically modified T cells in the patient's bloodstream to attack anti-CAR expressing malignant cells. Image adapted from (22).

cell therapies.[18,24] Tisagenlecleucel, also known as Kymriah, was the first CAR T therapy approved by the FDA on August 30, 2017, for the treatment of relapsed and refractory B-cell ALL and refractory diffuse large B-cell Lymphoma (DLBCL).[18,24] Axicabtagene ciloleucel, also known as Yescarta, is the second CD19-directed genetically modified T cell therapy approved by the FDA. It was approved on October 18, 2017, for the treatment of relapsed and refractory B-cell lymphomas and leukemias in adult patients.[18,24]

Each of the FDA approved anti-CD19 CARs contain either the CD28 or 4-1BB costimulatory domain interacting with the CD3-ζ chain.[19] Each of these domains induce a signal cascade that ultimately results in T cell activation, proliferation and necrotic/apoptotic effects on malignant cells.[19]

MECHANISMS OF ACTION

CD3 Zeta Chain

The multi-subunit TCR is composed of a TCR heterodimer, a ζ family homo-and heterodimer, and CD3 chains.[25] The CD3 chain is a protein encoded by the CD247 gene in humans and expressed on the T-lymphocyte surface. Activated TCRs induce tyrosine phosphorylation of the antigen recognition activation motif (ARAM) in the cytoplasmic domains of ζ chains and each of the CD3 chains.[25] After TCR activation, the cytoplasmic protein tyrosine kinase (PTK) ZAP-70 rapidly associates with the ζ and CD3 chains and undergoes tyrosine phosphorylation. The TCR-CD3 complex plays a key role in antigen recognition and transmembrane signalling.[26]

CD28 Co-Stimulation

CD28 increases TCR sensitivity by lowering the signalling threshold required for T cell activation.[19] CD28 contains a cytoplasmic tail composed of several motifs that initiates specific protein-protein interactions.[20] Alternative functions of the CD28 domain, specifically in the immunological synapse between T cells and CD19 antigen presenting cells (APCs), includes cytokine production, cell cycle progression, apoptosis, epigenetic structure modification and metabolism.[19] The activation of CD28 requires binding with ligands CD80 or CD86 expressed on APCs.[19,20] However, it is suggested that CD86 plays a greater role in initiation and CD80 in maintenance of immune responses.[19] CD28 co-stimulation is critical for IL-2 secretion and Bcl-X_L expression by the recruitment of the P13K/Akt pathway that has been previously activated by a series of signalling proteins. Bcl-X_L is an anti-apoptotic protein that enhances cell survival whereas, IL-2 is a T cell cytokine necessary for proliferation.[20,27]

4-1BB Co-Stimulation

4-1BB is a transmembrane protein expressed on activated T cells and APCs 4-1BBL is expressed on APCs which binds to 4-1BB to induce T cell responses via the tumour TNF-associated factors, TRAF 1 and 2.[19] This results in the subsequent activation of NF-kB, AKT, p38 MAPK and ERK pathways.[28] Through the previously mentioned pathways, 4-1BB signalling enhances T cell proliferation, cell cycle progression, cytokine secretion, cytolytic potential and resistance to transforming growth factor suppression.[19] This co-stimulation-mediated protein increases IFN and IL-2 secretion by $CD8^+$ T cells via T-helper cells (Th4), and IL-2 and IL-4 secretion by $CD4^+$ T cells.[19,29] IL-2 is a critical component for the growth and death factor of antigen activated T lymphocytes.[19,29] Alternatively, IL-4 serves as autocrine growth and differentiation factors, resulting in the proliferation and differentiation of T cells into effector cells.[30]

CLINICAL TRIALS

Pre clinical trials of CAR T began in 2002 which provided early indications of CAR T efficacy.[31,32]

Table 1 outlines the stages of clinical trials for both Yescarta and Kymriah. The trials for Yescarta are currently active for ZUMA-2,3 and 4, however the therapy was approved by the FDA on October 18, 2017, based on early results from the ZUMA-1 trial.[33] Kymriah was approved on May 1, 2018, based on the JULIET trial, however, the ELIANA trial was underway during the approval process as well.[34]

The FDA approval of Yescarta and Kymriah CAR T cell therapy for the treatment of R/R B-cell malignancies was dependent on the results of the final stages of the clinical trials (Table 1). Based on the results of the ZUMA-1 trials for Yescarta, 83 % of the 108 CAR T cell infused patients indicated a degree of complete remission, with no deaths induced by the immunotherapy.[36,38] The following phases of the ZUMA trial (2 to 4) could not be analyzed since these trials are currently underway.

On the other hand, Kymriah FDA approval was attributed to the JULIET and ELIANA trials. The JULIET clinical trial results showed complete remission in 95% of patients after 3 months of CAR T cell infusion.[43] The death of three patients in the clinical trial was not attributed to Kymriah administration, or Cytokine-Release Syndrome (CRS) and neurological events caused by the therapy.[43] Additionally, the ELIANA trials demonstrated complete remission in 60% of infused patients. The high rates of remission in the trials of Yescarta and Kymriah have demonstrated the effectiveness of anti-CD19 CAR T cells against B cell malignancies in pediatric and adult patients.[43] The re-

sults of these clinical trials reveal the dramatic efficacy of CD-19-targeted CAR T cells which have induced complete remission in up to 90% of patients with relapsed/refractory B-ALL, that would have otherwise had an expected response rate of 30% with chemotherapy.[46] However, this success is accompanied by adverse effects, including cytokine release syndrome and neurotoxicity.[43] Post marketing studies have been conducted to assess long term safety and risks of secondary malignancies. Numerous studies continue to explore new ways of minimizing the side effects accompanying CAR T cell therapy. With the potential to mitigate these side effects, the applications of CAR T therapy for the treatment of alternative forms of cancer is endless.

Table 1. Summary of clinical trials leading to the FDA approval of Yescarta and Kymriah.

FDA Approved CAR T Therapy	Clinical Trial	Patient Criteria	Implications of the Study	Results of the Clinical Trial
Yescarta	ZUMA- 1	Involved 108 patients 18 years or older, with relapsed/refractory (R/R) large B-cell lymphoma.[35] All patients had previously undergone anti-CD20 monoclonal antibody treatment and an anthracycline-containing chemotherapy.[35]	This trial took place in 22 academic centers administering lymphodepleting chemotherapy drugs cyclophosphamide and fludarabine at least 3 days prior to the administration of an infusion dose of 2 x 10^6 viable CAR T cells/ kg body weight.[35]	Two treatment related deaths occurred during the initial treatment but no deaths were reported after.[36] Grade >3 serious adverse events were reported in 48% of patients, with 11% experiencing severe cytokine release syndrome (CRS).[36,39] Follow up data collected from all 108 patients found that 83% had an objective response after 27 months indicating a degree of disease regression.[39]
	ZUMA-2	Involved 130 participants with R/R mantle cell lymphoma who were 18 years or older.[37] Patients must have been previously treated with anthracycline or bendamustine-containing chemotherapy, anti-CD20 monoclonal antibody therapy as well as Ibrutinib.[37]	This trial took place in 19 academic centres, administering lymphodepleting chemotherapy drugs cyclophosphamide and fludarabine at least 3 days prior to administration of a single infusion dose of 2 x 10^6 viable CAR T cells/ kg body weight.[37,38]	Results are not currently available as the trial is ongoing.[37]
	ZUMA-3	Involved 100 participants with R/R B-ALL over the age of 18. Patients were previously treated with blinatumomab, CD19 tumour expression in bone marrow or peripheral blood.[40]	Administration of fludarabine and cyclophosphamide provided prior to intravenous injection of 2 x 10^6 cells/kg body weight or 1 x 10^6 cell/kg body weight.[40]	Results are not currently available as the trial is ongoing.[37]
	ZUMA- 4	Involved 100 participants with R/R B-ALL between the ages of 2 and 21.[41,42] Patients must have previously been treated with 2 or more lines of chemotherapy or have refractory disease after stem cell transplant (SCT).[41,42]	Administration of fludarabine and cyclophosphamide provided prior to intravenous injection of 2 x 10^6 cells/kg body weight or 1 x 10^6 cell/kg body weight.[41]	Results are not currently available as the trial is ongoing.[38]
Kymriah (CTL019)	JULIET	Involved 99 patients, 18 years or older, with R/R diffuse large B-cell lymphoma (DLBCL).[43] The median number of prior lines of antineoplastic therapy was 3; 47% of patients had prior auto SCT. The median age was 56 years, 77% of those patients diagnosed with stage III or IV disease at study entry.[43]	This trial took place at 27 study centers in 10 countries on 4 continents .[43] Prior to infusion, patients underwent restaging and 93% received lymphodepleting chemotherapy.[43]	86% of patients had grade 3 or 4 adverse events (AEs); CRS occurred in 58% of infused patients.[43] Three patients died within 30 days of infusion, due to disease progression.[43] No deaths were attributed to CTL019, CRS or neurological events.[43] CTL019 produced high response rates with 95% of complete remissions (CRs) at 3 months being sustained at 6 months in a cohort of highly pretreated adult patients.[43]
	ELIANA	Single-arm, open-label trial (all subjects are informed of study components) enrolled 92 patients, of which 75 patients were infused between the age of 3 and 23 years with R/R B-cell ALL. 8% of the participants had primary refractory disease.[44] All patients received a median of 3 prior lines of therapy: 53% received a SCT, and 8% received 2 SCTs.[44]	This trial took place in 25 centers in 11 countries.[44]	After a 13.1 month follow up, the overall remission rate was 81%. 60% of patients achieved CR and 21% of patients achieved complete remission with incomplete blood count recovery.[44,45] All infused patients with best overall response of complete remission were minimal residual disease (MRD) negative, 95% by day 28. 77% of patients experienced grade 3 or grade 4 CRS. In this trial 25 deaths were reported after CAR-T infusion.[44,45]

MITIGATING INHERENT SIDE EFFECTS

The most common side effects of CAR T therapy across many clinical trials is Cytokine-Release Syndrome (CRS) and neurological toxicities. CRS is a systemic inflammatory response triggered by elevated cytokine release, known as a cytokine storm, as well as increased T cell activation and proliferation, shown in Figure 3.[45] Currently, there are multiple grading systems to identify and clinically treat various stages of CRS. Davila et al. (2014) suggests using IL-6 protein levels as an analogous measurement of antibody levels, where an increase in either protein concentration is an indication of an inflammatory immune response.[47] Implementing a standardized monitoring system across all international treatment centres could help to optimize treatment methodology and improve the quality of life of patients.[47] Neurological toxicity is generally viewed as a secondary outcome to CRS although the mechanism by which this occurs is not understood.[48,49] Burdno et al. (2016) hypothesized that as IL-6 levels increase, this protein serum congregates in the brain and causes secondary toxicity effects.[48] Furthermore, a second hypothesis suggested by Rooney et al. (2018) includes IL-6 as a cause of CRS, but demonstrates IL-1 to be the cytokine that specifically causes neurological toxicity (Figure 3).[50]

In a foundational study conducted by Maude et al. (2014), 100% of patients treated with CAR T CD19 therapy experienced CRS symptoms, 27% of which experienced severe symptoms.[51] Symptoms of mild CRS commonly include fever, hypotension, rapid heart rate as a result of hypoxia, organ dysfunction, and rashing.[51] Mild to moderate CRS generally occurs within 4 days of the first infusion and is usually monitored for the length of the entire study.[53] Severe and life-threatening CRS symptoms reported in Yescarta clinical studies included cardiac arrhythmias, renal insufficiency, cardiac arrest or failure, capillary leak syndrome and macrophage activation syndrome.[52]

The immunosuppressant, Tocilizumab, is FDA approved for the treatment of severe CRS due to its function as an IL-6 receptor antagonist.[51] For both FDA approved CAR-T immunotherapies, Kymriah and Yescarta cite CRS as the most common side effect and recommend tocilizumab as a first response to any serious CRS symptoms.[48,52]

Neurological toxicities often occur concurrently to CRS, causing mild to severe toxicity symptoms. Mild forms include dizziness, confusion and delayed verbal response, which generally manifest within the first 4 days after infusion and subside within 8 weeks of CAR T therapy initiation.[54,55] More severe side effects include global encephalopathy, hallucinations, delirium, cognitive defects, seizures and cerebral edema.[52] A study conducted by Burdno et al. (2016) hypothesized that Tocilizumab cannot cross the blood-brain barrier due to its large molecular size.[48] As a result, corticosteroids are generally administered as a first line response to symptoms of neurological toxicity because the molecular structure and charge allows it to permeate the blood-brain barrier.[48,49]

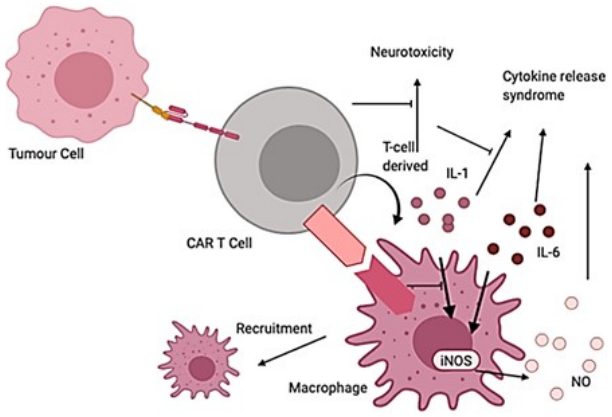

Figure 3. A simple model for CRS depicting the interaction between CAR T cells with tumour cells as well as macrophages. The macrophage interaction activates the release of IL-6 and IL-1 chemokines, which further activates the innate immune response and exacerbates the inflammatory signals causing CRS and neurotoxicity. Image adapted on BioRender.[53]

FUTURE CONSIDERATIONS—VIABILITY FOR SOLID TUMOURS

Overcoming Microenvironment and Immune Suppression

The microenvironment created by solid tumours presents many challenges for immune modulating therapies. One way to mitigate this challenge is by the introduction of a fourth generation CAR T cell that is "armoured".[56] These cells are engineered to withstand the toxic microenvironment by coupling the antitumour effect of CAR T with the secretion of IL-2 or IL-12.[56] IL- 12 is a heterodimeric cytokine produced by the activation of inflammatory cells such as T lymphocytes and natural killer (NK) cells.[57] To prevent toxicity associated with constitutive IL-12 production, a study by Zhang et al. (2011) attempted to create an inducible promoter for transcribing the protein.[57] To do so, an inducible retroviral vector was developed alongside a nuclear factor of activated T cells (NFAT)-responsive promoter to restrict IL-12 expression to specific tumour antigen recognition. Although this is the first study that has attempted to control IL-12 expression in this manner, the use of this method demonstrates promising results and is thereby continuing to be explored.[57] This modification is the beginning for establishing an "on and off" mechanism for future immune modulating therapies.[58]

A fundamental limitation to immunotherapies for solid-tumour cancers is the lack of adaptation to heterogeneity at the immunological level of the tumour lesion.[59] Antigen specific targeting provides powerful and long lasting effects against tumour cells, however, only on a very small scope of cells at a given time.[59] One way to navigate the restrictive nature of this therapy is to potentiate the endogenous immune system, suppressed by the tumour, to recognize and destroy cells not directly targeted by the CAR-T therapy.[57,59] IL -12 secretion can recruit and reinforce innate macrophage function, thereby encouraging the detection and destruction of antigen lacking cancer cells that would not have been otherwise detected by the therapy itself.[57,59] This accumulation of macrophages also contributed to a sustained antitumor response.[57] Thus, the induction of IL-12 provides access to otherwise inaccessible tumour regions as these cells are undetectable using the antigen targeting method of CAR T and provide a proinflammatory response.[58] Antigen-lacking cells are partially responsible for the progression of tumours despite treatment with antigen specific therapies, as they are able to evade the modified T cells and continue to proliferate and increase the strength of the tumour and the microenvironment it creates.[60] Since this proposed process would occur with the patient's innate immune system, the toxicity associated with this IL-12 catalyzed process is theoretically far less than direct CAR T binding and subsequent cytokine release.[59] More research is required to find other potential immunostimulatory cytokines to create an immunological memory and independently suppress the reoccurrence of tumours using the innate immune response as a first line of defense.[60]

Overcoming Physical Barriers

There are also physical barriers that must be mitigated. Specifically, when treating solid tumours present in the epithelial and mesenchymal compartments which are not significant in hematopoietic cancers.[55] One study by Pegram et al. (2014) has shown that engineered T cells may not currently be equipped with the tools required to break through these outer barriers compared to natural immune cells.[60] The trafficking of immune cells towards the tumour foci is significantly inhibited by the overexpression of ligands and receptors on the tumour endothelium.[60] T lymphocytes are able to degrade the extracellular matrix specifically the heparan sulphate proteoglycans (HSPGs) component during extravasation.[55] Natural T cells do this by expressing heparanase (HPSE) to degrade the HSPGs and access the tumour cells.[55] Recent studies show that HPSE mRNA is downregulated in *in vitro*-expanded T cells, therefore by engineering cells to express or overexpress this enzyme, CAR T cells may be able to infiltrate further into stroma-rich solid tumours and decrease the barriers associated with solid tumour contact.[55]

BUDGET PLAN AND TREATMENT OPTIONS

Although CAR T therapy has shown promising results for the treatment of R/R B-ALL, it is critical to note the accessibility of this new innovative immunotherapy to the public. In order to determine the most beneficial treatment plan for a patient suffering from ALL, it is important to consider all treatment options, while concurrently observing the financial burden each treatment would impose on the individual.

Currently, the cost of CAR T therapy in the United States ranges from $373,000 to $475,000 for Yescarta and Kymriah, respectively.[61] The high estimated cost of these therapies does not include hospital stays and extended treatments for patients who experience CRS or other adverse effects. It is important to note that there is no information available for CAR T therapy in Canada as it has only been conditionally approved by Health Canada since September 5, 2018. However, it is not available to Canadian residents yet as several components of this decision need to be finalized. Currently, Health Canada offers coverage for patients 3-25 years of age.[61] This however does not specify coverage for leukapheresis, or other essential techniques associated with the therapy. Negotiations are ongoing between Novartis and Canadian Cancer Care Ontario, with plans to reduce the manufacturer cost by at least half before government coverage is offered to the public.[62] Using the assumption that the procedures associated with the CAR T treatment are not covered under this negation, the cost should be minimal. For example, the leukapheresis procedure necessary to extract the white blood cells will cost approximately $550 CDN.[63] This estimate is based on the current rate of plasma exchange procedures which is another type of apheresis treatment that requires similar equipment and treatment costs. Currently, Kymriah's only trial centre in Canada is located at the SickKids hospital in Toronto, therefore creating an additional cost for those that live out of province.[64]

Based on the cost analysis of the current treatment options, there are many financial implications that accompany these treatments for patients with B-ALL, apart from the therapy itself.[65] These include hospital stays, therapy cost, ongoing physician care and transportation costs if the treatment is only offered at specific institutions.[66,67] The standardized treatment options for B-ALL are chemotherapy, radiation therapy, or stem cell transplants.[67] Of these three options, chemotherapy and radiation therapy require multiple visits by patients undergoing this particular treatment plan. Based on this, it is important to consider that the individual would experience higher hospital care and transportation costs, compared to the same patient undergoing a stem cell transplant or CAR T therapy. Stem cell transplants usually require patients to remain in the hospital for a period of 2-6 weeks until the

patient's blood cell count returns back to normal levels after transplantation.[67,68] In contrast, CAR T therapy patients usually stay in the hospital for at least 7 days after receiving treatment, and are required to stay within 2 hours travel time of the hospital for regular follow ups until at least 4 weeks after leaving the hospital.[69] The cost to stay in a location within this transportation time range is an additional cost to consider, however, the Canadian Cancer Society offers cancer lodges that are available to cancer patients with a lower price for overnight stay, including meals.[70] It is important to note that chemotherapy treatment can be 4-6 months in length.[68] With this treatment option in mind, transportation, extended stay near the treatment centre, and other additional costs which can extend over a relatively long period of time can result in accumulating financial burden. In addition to the primary cost of all treatment modalities, secondary costs associated with hospital stay must be accounted for when analyzing treatment options. For example, radiation therapy is administered in fractions lasting approximately 5-8 weeks, with the number of fractions depending on the treatment plan.[71] Although this is an outpatient service, daily radiation treatments require additional costs similar to those for chemotherapy and stem cell transplant.[36]

The present cost of CAR T therapy is out of reach for those without coverage or adequate personal funding. Therefore, it is not recommended for patients who have access to less expensive options. Due to the high remission rate demonstrated by clinical trials, CAR T is an effective option for patients who have not seen success with the traditional treatments, in which case CAR T would be recommended based solely on successful remission rate. Overall, CAR T therapy is extremely effective, and is recommended for those who can afford it at its current price. Every cancer treatment has the potential to be a large financial burden on the patient and their family, therefore it is necessary to plan for each unique case. CAR T therapy has promising advancements in the future as a cancer treatment and could one day become a main treatment modality.

CONCLUSION

B-ALL is an aggressive leukemia subtype with wide genetic variation requiring individualized treatment. Current treatment options include general systemic chemotherapy and combined therapeutic treatments. By utilizing the body's immune system, a greater level of personalized medicine can be achieved as the procedure of CAR T therapy retrieves and modifies T cells directly from the patient. The CD19 target in particular has shown great promise since it is only expressed on malignant B cells in B-ALL. Additionally, both of the current FDA approved treatments; Kymriah and Yescarta utilize the CD19 target. Several clinical trials including those for Kymriah and Yescarta have shown an improved safety profile and remission rate. The side effects caused by these therapies can be severe such as neurotoxicity and CRS, however there are now many techniques to mitigate adverse effects to ensure patient safety. The next step for CAR T therapy will be to overcome the physical and physiological barriers associated with solid tumours, in order to become a viable treatment option for solid tumour cancers in the future. CD19-targeted CARs have paved the way for engineered T cell therapies with high response rates against relapsed and refractory B-cell malignancies. Although CAR T has shown beneficial responses in highly refractory populations, there are several limitations that must be considered.[71,72] The complex preparation, economic factors, lack of accessibility in certain countries (ie. Canada), and the inability to successfully treat solid tumours complicates the potential for this therapy to reach a broader public. As CAR T cell technology continues to develop, there is the potential for the discovery of a more viable, affordable CAR T treatment option with improved safety and efficacy.

ACKNOWLEDGEMENTS

This work did not receive funding. There are no conflicts of interests.

REFERENCES

(1) NIH. NCI Dictionary of Cancer Terms [Internet]. National Cancer Institute. 2011 [cited 2020Feb20]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms

(2) Pui C-H, Evans WE. Treatment of Acute Lymphoblastic Leukemia. New England Journal of Medicine. 2006Dec;354(2):166–78.

(3) Paul S, Kantarjian H, Jabbour EJ. Adult Acute Lymphoblastic Leukemia. Mayo Clinic Proceedings. 2016;91(11):1645–66.

(4) NCCN. NCCN Guidelines for Patients® | Acute Lymphoblastic Leukemia [Internet]. National Comprehensive Cancer Network. [cited 2020Feb19]. Available from: https://www.nccn.org/patients/guidelines/all/index.html

(5) Pui C-H, Evans WE. Treatment of Acute Lymphoblastic Leukemia. New England Journal of Medicine. 2006Dec;354(2):166–78.

(6) Klein AD, Kessel AGV, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300(5894):765–7.

(7) National Institute of Health. Adult Acute Lymphoblastic Leukemia Treatment. National Cancer Institute [Internet]. cancer.gov. 2020. Available from: https://www.cancer.gov/types/leukemia/hp/adult-all-treatment-pdq

(8) Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood. 2002Jan;99(3):863–71.

(9) Hallböök H, Simonsson B, Ahlgren T, Björkholm M, Carneskog J, Grimfors G, et al. High-dose cytarabine in upfront therapy for adult patients with acute lymphoblastic leukaemia. British Journal of Haematology. 2002;118(3):748–54.

(10) Kantarjian H, Thomas D, Obrien S, Cortes J, Giles F, Jeha S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. Cancer. 2004;101(12):2788–801.

(11) Nguyen K, Devidas M, Cheng S-C, La M, Raetz EA, Carroll WL, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Childrens Oncology Group study. Leukemia. 2008;22(12):2142–50.

(12) Lee S, Kim Y-J, Min C-K, Kim H-J, Eom K-S, Kim D-W, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome–positive acute lymphoblastic leukemia. Blood. 2005Jan;105(9):3449–57.

(13) Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children

with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001Jan;97(5):1211–8.

(14) Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. Blood. 2004Dec;103(12):4396–407.

(15) Schrappe M, Reiter A, Ludwig W-D, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. Blood. 2000;95(11):3310-3322. http://www.bloodjournal.org/content/95/11/3310.Accessed March 30, 2019.

(16) Hunault M, Harousseau J-L, Delain M, et al. Better outcome of adult acute lymphoblastic leukemia after early genoidentical allogeneic bone marrow transplantation (BMT) than after late high-dose therapy and autologous BMT: a GOELAMS trial. Blood. 2004;104(10):3028–37.

(17) Sadelain M, Brentjens R, Rivière I. The Basic Principles of Chimeric Antigen Receptor Design. Cancer Discovery. 2013;3(4):388–98.

(18) Miliotou AN, Papadopoulou LC. CAR T cell Therapy: A New Era in Cancer Immunotherapy. Current Pharmaceutical Biotechnology. 2018;19(1):5–18.

(19) Sjoukje J. C. Van Der Stegen, Hamieh M, Sadelain M. The pharmacology of second-generation chimeric antigen receptors. Nature Reviews Drug Discovery. 2015;14(7):499–509.

(20) Boomer JS, Green JM. An Enigmatic Tail of CD28 Signaling. Cold Spring Harbor Perspectives in Biology. 2010Sep;2(8).

(21) Goodsell D. Protein Data Bank - Molecule of the Month: Chimeric Antigen Receptors [Internet]. RCSB. [cited 2020Feb20]. Available from: https://pdb101.rcsb.org/motm/214

(22) LaRussaA. Chimeric Antigen Receptor (CAR) T cell Therapy [Internet]. Leukemia and Lymphoma Society. 2015 [cited 2020Feb20]. Available from: https://www.lls.org/treatment/types-of-treatment/immunotherapy/chimeric-antigen-receptor-car-T cell-therapy

(23) Boston MA. How CAR T cell Therapy Works [Internet]. Dana-Farber Cancer Institute. 2019 [cited 2020Feb20]. Available from: https://www.dana-farber.org/cellular-therapies-program/car-T cell-therapy/how-car-T cell-therapy-works/

(24) Yescarta (axicabtagene ciloleucel) FDA Approval History [Internet]. Drugs.com. [cited 2020Feb20]. Available from: https://www.drugs.com/history/yescarta.html.

(25) Iwashima M, Irving B, Oers NV, Chan A, Weiss A. Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases. Science. 1994;263(5150):1136–9.

(26) Klausner RD, Weissman AM, Baniyash M, Bonifacino JS, Samelson LE. The Role of the Zeta Chain in the Expression, Structure and Function of the T Cell Receptor. In: Gupta S, Paul WE, editors. Mechanisms of Lymphocyte Activation and Immune Regulation II. Boston, MA: Springer US; 1989 [cited 2020 Mar 12]. p. 21–4. Available from: http://link.springer.com/10.1007/978-1-4757-5803-0_3

(27) Fiebig AA, Zhu W, Hollerbach C, Leber B, Andrews DW. Bcl-XL is qualitatively different from and ten times more effective than Bcl-2 when expressed in a breast cancer cell line. BMC Cancer [Internet]. 2006 Dec [cited 2020 Feb 19];6(1). Available from: http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-6-213

(28) Nam K-O, Kang H, Shin S-M, Cho K-H, Kwon B, Kwon BS, et al. Cross-Linking of 4-1BB Activates TCR-Signaling Pathways in CD8 + T Lymphocytes. The Journal of Immunology [Internet]. 2005 Feb 15 [cited 2020 Feb 19];174(4):1898–905. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.174.4.1898

(29) Malek TR. The main function of IL-2 is to promote the development of T regulatory cells. Journal of Leukocyte Biology. 2003 Dec [cited 2020 Feb 19];74(6):961–5. Available from: http://doi.wiley.com/10.1189/jlb.0603272

(30) Choi P, Reiser H. IL-4: role in disease and regulation of production. Clin Exp Immunol.1998Sep;113(3):317–9. Availablefrom:https://doi.org/10.1046/j.1365-2249.1998.00690.x

(31) Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ /CD28 receptor. Nature Biotechnology. 2002 Jan [cited 2020 Feb 19];20(1):70–5. Available from: http://www.nature.com/articles/nbt0102-70

(32) Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric Receptors Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy In Vivo. Molecular Therapy [Internet]. 2009 Aug [cited 2020 Feb 19];17(8):1453–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001616318688

(33) FDA. Comissioner O of the. Press Announcements- FDA Approves CAR-T cell therapy to treat adults with certain typed of large B-cell lymphoma [Internet]. Available from: ucm581216.htm.Accessed March 31, 2019.

(34) National Cancer Institute. Tisagenlecleucel Approved to Treat Some Lymphomas [Internet]. National Cancer Institute. 2018. Available from: https://www.cancer.gov/news-events/cancer-currents-blog/2018/tisagenlecleucel-fda-lymphoma

(35) Federal Drug Administration. Highlights of Prescribing Information- Yescarta [Internet]. 2017. Available from: https://www.fda.gov/downloads/UCM581226.pdf

(36) Locke FL, Neelapu SS, Bartlett NL, Siddiqi T, Chavez JC, Hosing CM, et al. Phase 1 Results of ZUMA-1: A Multicenter Study of KTE-C19 Anti-CD19 CAR T Cell Therapy in Refractory Aggressive Lymphoma. Molecular Therapy [Internet]. 2017 Jan [cited 2020 Feb 19];25(1):285–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S152500161645375X

(37) Katz, Ariela. Investigating the Potency of CAR T cell Therapy in Relapsed/Refractory MCL [Internet]. Oncology Nursing News. 2018. Available from: https://www.oncnursingnews.com/publications/oncology-nurse/2018/october-2018/investigating-the-potency-of-car-T cell-therapy-in-relapsed-refractory-mcl

(38) NIH. A Phase 2 Multicenter Study Evaluating Subjects With Relapsed/Refractory Mantle Cell Lymphoma [Internet]. ClinicalTrials.gov. 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT02601313

(39) Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. The Lancet Oncology [Internet]. 2019 Jan [cited 2020 Feb 19];20(1):31–42. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1470204518308647

(40) NIH. A Study Evaluating KTE-X19 in Adult Subjects With Relapsed/Refractory B-precursor Acute Lymphoblastic Leukemia (ZUMA-3) [Internet]. ClinicalTrials.gov. 2019. Available from: https://clinicaltrials.gov/ct2/show/NCT02614066

(41) NIH. Study Evaluating KTE-C19 in Pediatric and Adolescent Subjects With Relapsed/Refractory B-precursor Acute Lymphoblastic Leukemia [Internet]. Clinical Trials NIH. 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT02625480

(42) Wayne AS, Sender LS, Lee DW, Handgretinger R, Brown PA, Jiang Y, et al. ZUMA-4: A phase 1/2 multicenter study evaluating the safety and efficacy of KTE-C19 (anti-CD19 CAR T cells) in pediatric and adolescent subjects with relapsed/refractory B-precursor acute lymphoblastic leukemia (r/r ALL). Journal of Clinical Oncology [Internet]. 2016 May 20 [cited 2020 Feb 19];34(15_suppl):TPS7075–TPS7075. Available from: http://ascopubs.org/doi/10.1200/JCO.2016.34.15_suppl.TPS7075

(43) Schuster SJ, Bishop MR, Tam CS, et al. Primary Analysis of Juliet: A Global, Pivotal, Phase 2 Trial of CTL019 in Adult Patients with Relapsed or Refractory Diffuse Large B-Cell Lymphoma. Blood. 2017;130(SUPP 1):577–577. Available from: http://www.bloodjournal.org/content/130/Suppl_1/577

(44) Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. New England Journal of Medicine. 2018 Feb [cited 2020 Feb 19];378(5):439–48. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1709866

(45) ASCO,2019. ASH 2018: ELIANA Trial: Tisagenlecleucel in Pediatric and Young Adult Patients With ALL - The ASCO Post. [Internet]. Available from: <http://www.ascopost.com/News/59526http://www.ascopost.com/News/59526>

(46) Davila ML, Brentjens RJ. CD19-Targeted CAR T Cells as Novel Cancer Immunotherapy for Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia. Clinical Advancements in Hematology Oncology. 2016Oct;14(10):802–8.

(47) Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. Science Translational Medicine [Internet]. 2014 Feb 19 [cited 2020 Feb 19];6(224):224ra25-224ra25. Available from: https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.3008226

(48) Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood [Internet]. 2016 Jun 30 [cited 2020 Feb 19];127(26):3321–30. Available from: https://ashpublications.org/blood/article/127/26/3321/35340/Toxicities-of-chimeric-antigen-receptor-T cells

(49) Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T cell therapy. Molecular Therapy - Oncolytics. 2016 [cited 2020 Feb 19];3:16011. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2372770516300353

(50) Rooney C, Sauer T. Modeling cytokine release syndrome. Nature Medicine]. 2018 Jun [cited 2020 Feb 19];24(6):705–6. Available from: http://www.nature.com/articles/s41591-018-0068-9

(51) Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia. Science Translational Medicine. 2013 Mar 20 [cited 2020 Feb 19];5(177):177ra38-177ra38. Available from: https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.3005930

(52) NIH. NCI Dictionary of Cancer Terms [Internet]. National Cancer Institute. 2011. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms

(53) Lymphoma Research Foundation. Diffuse Large B-Cell Lymphoma: Relapsed/Refractory [Internet]. [cited 2020Feb20]. Available from: https://www.lymphoma.org/aboutlymphoma/nhl/dlbcl/relapseddlbcl/

(54) Brudno JN, Kochenderfer JN. Recent advances in CAR T cell toxicity: Mechanisms, manifestations and management. Blood Reviews [Internet]. 2019 Mar [cited 2020 Feb 19];34:45–55. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268960X18300705

(55) Caruana I, Savoldo B, Hoyos V, Weber G, Liu H, Kim ES, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lympho-

cytes. Nature Medicine [Internet]. 2015 May [cited 2020 Feb 19];21(5):524–9. Available from: http://www.nature.com/articles/nm.3833

(56) Zhang L, Kerkar SP, Yu Z, Zheng Z, Yang S, Restifo NP, et al. Improving Adoptive T Cell Therapy by Targeting and Controlling IL-12 Expression to the Tumor Environment. Molecular Therapy. 2011 Apr [cited 2020 Feb 19];19(4):751–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001616304312

(57) LaMotta, Lisa. Gene therapy heavyweights talk next steps for CAR-T [Internet]. BioPharma Dive. Available from: https://www.biopharmadive.com/news/gene-therapy-heavyweights-talk-next-steps-for-car-t/521125/

(58) Chmielewski M, Kopecky C, Hombach AA, Abken H. IL-12 Release by Engineered T Cells Expressing Chimeric Antigen Receptors Can Effectively Muster an Antigen-Independent Macrophage Response on Tumor Cells That Have Shut Down Tumor Antigen Expression. Cancer Research. 2011 Sep 1 [cited 2020 Feb 19];71 (17):5697–706. Available from: http://cancerres.aacrjournals.org/cgi/doi/10.1158/0008-5472.CAN-11-0103

(59) Gilham DE, Debets R, Pule M, Hawkins RE, Abken H. CAR–T cells and solid tumors: tuning T cells to challenge an inveterate foe. Trends in Molecular Medicine [Internet]. 2012 Jul [cited 2020 Feb 19];18(7):377–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S147149141200072X

(60) Pegram HJ, Park JH, Brentjens RJ. CD28z CARs and Armored CARs: The Cancer Journal [Internet]. 2014 [cited 2020 Feb 19];20(2):127–33. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00130404-201403000-00007

(61) Hernandez I, Prasad V, Gellad WF. Total Costs of Chimeric Antigen Receptor T - Cell Immunotherapy. JAMA Oncology. 2018 Jul 1 [cited 2020 Feb 19];4(7):994. Available from: http://oncology.jamanetwork.com/article.aspx?doi=10.1001/jamaoncol.2018.0977

(62) Kelly Grant. Gene therapy drug Kymriah should be publicly covered if manufacturer drops price, report recommends. The Globe and Mail [online] [Internet]. January. 2019; Available from: https://www.theglobeandmail.com/canada/article-gene-therapy-drug-kymriah-should-be-publicly-covered-if-manufacturer/

(63) Rock, G., Clark, B. and Sutton, D. The Canadian apheresis registry. Transfusion and Apheresis Science. 2003;29(2):167–77. Available from: http://www.sciencedirect.com/science/article/pii/S1473050203001113

(64) Canadian Agency for Drug and Technologies in Health. Tisagenlecleucel for Acute Lymphoblastic Leukemia and Diffuse Large B-cell Lymphoma: Recommendations. Canadian Agency for Drug and Technologies in Health. 2019;8(3):18.

(65) Kaul S, Korgenski EK, Ying J, Ng CF, Smits-Seemann RR, Nelson RE, et al. A retrospective analysis of treatment-related hospitalization costs of pediatric, adolescent, and young adult acute lymphoblastic leukemia. Cancer Medicine [Internet]. 2016 Feb [cited 2020 Feb 19];5(2):221–9. Available from: http://doi.wiley.com/10.1002/cam4.583

(66) Canadian Cancer Society. Treatments for childhood ALL [Internet]. Canadian Cancer Society. 2019. Available from: http://www.cancer.ca/en/cancer-information/cancer-type/leukemia-childhood/treatment/acute-lymphocytic-leukemia-all/?region=on#Planning

(67) Canadian Cancer Society. After the stem cell transplant [Internet]. www.cancer.ca. Available from: http://www.cancer.ca/en/cancer-information/diagnosis-and-treatment/stem-cell-transplant/after-stem-cell-transplant/?region=on

(68) Chemocare. How Long is Chemotherapy Given? - What is Chemotherapy? [Internet]. Chemocare. 2019. Available from: http://chemocare.com/chemotherapy/what-is-chemotherapy/how-long-is-chemotherapy-given.aspx

(69) Finney HM, Lawson ADG, Bebbington CR, Weir ANC. Chimeric Receptors Providing Both Primary and Costimulatory Signaling in T Cells from a Single Gene Product [Internet]. The Journal of Immunology. American Association of Immunologists; 1998 [cited 2020Feb20]. Available from: http://www.jimmunol.org/content/161/6/2791

(70) Cancer Care Society. Travel and accommodation [Internet]. cancer.ca. Available from: http://www.cancer.ca/en/support-and-services/support-services/travel-and-accommodations-on/?region=on

(71) American Cancer Society, 2017. *External Beam Radiation Therapy*. [online] American Cancer Society. Available at: <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/radiation/external-beam-radiation-therapy.html> [Accessed 7 Apr. 2019].

(72) Xu X, Sun Q, Liang X, Chen Z, Zhang X, Zhou X, et al. Mechanisms of Relapse After CD19 CAR T cell Therapy for Acute Lymphoblastic Leukemia and Its Prevention and Treatment Strategies. Frontiers in Immunology. 2019 Nov 12 [cited 2020 Feb 19];10. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.02664/full