Journal of Oncological Sciences 4 (2018) 134–141

Contents lists available at ScienceDirect

Journal of Oncological Sciences

journal homepage: https://www.elsevier.com/locate/jons

Review

Cytokine release syndrome

Esra Yildizhan [a], Leylagul Kaynar [b, *]

[a] Kayseri Education and Research Hospital, Hematology Department, Kayseri, Turkey
[b] Erciyes University, Faculty of Medicine, Hematology Department, Kayseri, Turkey

ARTICLE INFO

Article history:
Received 17 July 2018
Accepted 30 September 2018
Available online 9 October 2018

Keywords:
Cytokine relase syndrome
Immunotherapy
Cell therapies
CAR T Cell

ABSTRACT

The field of immunotherapies including monoclonal antibodies and cellular therapies is rapidly evolving and cytokine release syndrome is an obstacle to deal with in this process. This syndrome is a kind of toxic condition caused by the pro-inflammatory cytokines released with the activation of the extreme immune response. In fact, up to some extent, the cytokine release may be an indicator of the antitumor effects of immune-based therapies. Therefore, the distinction between the level at which the efficiency of treatment is ensured and the point at which the life-threatening cytokine release syndrome starts is critically important. Recently, considerable developments have been achieved with regard to the prevention or control of this syndrome. In this review it is aimed to summarize the clinical approach to cytokine release syndrome and associated conditions.

New monoclonal antibodies (moAb) and cellular therapies with stronger anti-tumor effects are evolving every day and used particularly in the fields of oncology, hematology, and organ transplantation; however these developments are overshadowed by serious adverse effects such as CRS. According to 'The National Cancer Institute Common Terminology Criteria for Adverse Events' (NCI-CTCAEs) CRS is defined as a condition that is caused by an extreme immune response with excessive cytokine release causing symptoms such as nausea, headache, tachycardia, hypotension, rash, and dyspnea.[1] These cytokines are released by T and B lymphocytes, natural killer cells, monocytes and macrophages. They activate the inflammatory cascade causing uncontrolled endothelial damage resulting in a systemic inflammatory response with multiple organ dysfunction and even death as result.[2]

Clinical syndromes caused by immunotherapy can be based upon two different pathways. First is autoimmune toxicity; the targeted antigen is also presented by non-tumor tissues and might cause the destruction of healthy tissue. Second is cytokine-mediated toxicity; this toxic condition is caused by pro-inflammatory cytokines released due to activation of an extreme cellular immune response independent of the antigen. CRS is considered as an example of the latter.

MoAbs are known to cause this condition most frequently, even though it was not always specified as a syndrome but referred as an immune reaction, anaphylactic reaction, or adverse event. In 1981, it was first mentioned that administration of muromonab (okt3, anti-CD3), which is the first moAb that is currently being used after solid organ transplantation, caused increased cytokine levels and systemic reaction.[3] Subsequently severe adverse systemic reactions caused by murine moAb T101 and alemtuzumab (moAb against CD52, CAMPATH 1-H) have been reported.[4–7] Likewise, in a phase 1 study on TGN1412, an anti-CD28 moAb, CRS developed in all of the six patients after administration of TGN1412.[8] After this study in 1996, TGN1412 was no longer used as therapy in humans, although experimental studies continued.

Rituximab, a murine-human chimeric anti-CD20 moAb, is probably the most frequently used biological agent in hematologic malignancies today. Actually, clinicians' initial confrontation with this syndrome was by means of rituximab.

1. Rituximab and CRS

The mechanism of rituximab is based on complement mediated cell lysis and antibody dependent cellular toxicity. Although natural killer cells play major role in cellular toxicity, the interaction of the Fc of the anti-CD20 with the Fcγ receptor on macrophages contributes to cytokine release (FcγR/Fc mediated opsonic phagocytosis or cytotoxicity). In addition, it causes damage to the calcium channel of the CD20 antigen inducing apoptosis due to intracellular calcium overload. Lysis of these target cells can cause a dramatic increase of cytokines within minutes.[9,10] Infusion associated

* Corresponding author. Fax : +90 352 207 6666
E-mail address: lgkaynar@erciyes.edu.tr (L. Kaynar).
Peer review under responsibility of Turkish Society of Medical Oncology.

https://doi.org/10.1016/j.jons.2018.09.002

adverse effects appear in approximately 25% of the patients after administration of rituximab. Although it is reported that most of these reactions can easily be treated, this remains controversial. As well, some fatal events are reported in some limited studies and case reports.[11] For example Makino et al. reported the death of a patient with lymphoma 5 h after rituximab administration most likely due to CRS.[12] Several factors play a role in infusion associated adverse effects of rituximab and the risk of developing CRS such as tumor burden, first or subsequent cycle of treatment, the dosage and infusion rate.[13] Hannawa et al. demonstrated that reducing tumor burden by conventional chemotherapy before rituximab can reduce the risk and severity of CRS by preserving the efficacy of therapy.[14]

Development of CRS is also a problem after the administration of cellular therapies including bispecific antibodies (blinatumomab, BiTE), haploidentical mononuclear cell infusion, and chimeric antibody expressing (CAR) T cell. These therapies are particularly promising in B cell acute lymphoblastic leukemia (B-ALL), non-Hodgkin lymphomas, and chronic lymphocytic leukemia (CLL).[15,16]

2. Cellular therapies and CRS

Blinatumomab is the first BiTE® molecule to have been approved by the US Food and Drug Administration (FDA) for relapsed/refractory Philadelphia negative B-ALL. In the initial clinical studies in patients with B cell malignancies, blinatumomab was poorly tolerated because of side effects including CRS and neurotoxicity.[17] In fact, the central nervous system (CNS) adverse events are considered to be associated with cytokine release induced by the targeted T cells that enter the CNS. In most of these studies CRS was usually seen in the first cycle of treatment and was related to high disease burden. The risk of CRS was lower for those in minimal residual disease. Recently, in a multicenter study on blinatumomab no adverse event related with CRS has been reported in patients with diffuse large B cell lymphoma by stepwise dosing and with dexamethasone prophylaxis.[18]

CAR T cell therapy is also particularly promising but there are many reports on CRS related with CAR T cell therapy, even some of them are fatal. (30–94%, including severe CRS 1–48%).[19–24] Recently, first anti CD19 CAR T cell product tisagenlecleucel (CTL019) was approved by FDA for treatment of patients younger than 25 years of age with relapsed or refractory B-cell precursor ALL. In current studies in this population CRS related with tisangenlecleucel is reported in 77–82% of the patients and 44–48% were severe that required anti-cytokine therapy.[25,26] These studies are mostly conducted on pediatric patients with ALL and it is known that the risk of toxicity related with conventional chemotherapy increases with patient age. Similarly, after CAR T cell therapy, older patients might experience CRS -or severe CRS- more than children. There is no much data in adults but in a study by Turtle et al. CRS was reported in 82% of the adult patients (28% of them were grade3-4) with B-ALL who received CD19 CAR T cell therapy after lymphodepletion chemotherapy including cyclophosphamide with or without fludarabine.[27] In this study 30 patients enrolled with a median age of 40 years (range 20–73) the rate of CRS was similar to those in pediatrics but the severe form of disease was less frequent and responded rapidly to the treatment. Authors emphasized that lymphodepletion -especially with fludarabine- and CAR T cell dosing strategies mitigate the toxicity.

The high rates of CRS mostly reported in studies with ALL patients. Indeed; in another study on CTL019 in adult patients with diffuse large B cell lymphoma (LULIET study) CRS occurred in 57% of the patients (17% grade 3; 9% grade 4); and there was no CRS-associated death.[21] Similarly, a CD19 CAR T cell product (CAR017) was studied in a phase 1 study on adult patients with B cell non-Hodgkin Lymphoma and CRS was reported in 30% of the patients (1% grade4).[23]

Another autologous anti-CD19 chimeric antigen receptor CAR T cell product axicabtagene ciloleucel (axi-cel, KTE-C19) was studied in patients with refractory B-cell lymphoma. Grade 3 or higher cytokine release syndrome occurred in 13% of the patients.[28] All of these reports revealed that tumor burden, underlying disease, CAR T cell dosage, use of lymphodepleting chemotherapy and also comorbidities and age of patient affect the development and management of CRS. Although some studies have mentioned the correlation between CRS and the efficiency of CAR T cell therapy, the effect on outcome was not found strong.[24]

In contrast to monoclonal antibodies, CRS may progress more severely in T cell therapies due to continuous cytokine release during the whole lifespan of the cells. Based on this information, studies on developing short living CAR T cells with suicide genes or mRNA are being conducted.[29] Despite all these studies, CRS still constitutes a serious problem for cellular therapies and other alternatives are needed for its prevention.

The risk of developing CRS depends on the characteristics of the given biological, the manufacturing, quantity, selection and activation of these immune cells, but also on patient characteristics such as the underlying disease, tumor burden -high number of lymphocytes-, lymphodepletion using cyclophosphamide and fludarabine before immunotherapy and probably also the genetic structure of the patients.[13] For instance, the maximum cytokine level is approximately 90 min after administration of rituximab whereas symptoms will appear in minutes to hours, although it might vary depending on the infusion rate.[13,20] For alemtuzumab, increase of cytokines ranges between 2 and 4 h.[6] For cellular therapy the onset of CRS toxicity usually occurs between 6 h and 10 days but the effect will last much longer due to the continuity of cell expansion.[30,31] Turtle et al. showed a direct relation between the number of administrated cells and CRS in CAR T cell therapy, they conducted the study on 3 dose level (2×10^5/kg; 2×10^6/kg; and 2×10^7/kg) and they reported fatal cases after giving high numbers of cells (2×10^7/kg cells). They emphasized that individualizing the dose of CAR T cells may provide optimal efficacy and safety. In patients with high tumor burden it would be better to keep the CAR T cell dose low to decrease the risk of CRS, whereas in patients with low tumor burden higher dose of CAR T cells may be required to ensure the adequate antigenic stimulation.[31]

Another variable that is thought to play a role in CRS is genetic polymorphism. It is already known that polymorphism in cytokine genes affect cytokine levels in sepsis and have a role in progression towards septic shock.[32] Morgan et al. identified the same genotypes, IL6-174 G/C and IL10-1082 G/A in a patient with CRS after CAR T cell therapy.[33] This finding may explain the heterogeneity of CRS in different patients.

3. Pathophysiology

In both monoclonal antibodies or cellular therapies, mediators that are considered to be important in developing CRS are interferon gamma (IFNγ), IL6, and tumor necrosis factor alpha (TNFα) as well as interleukin1 beta (IL1β), IL2, IL10, IL8, IL5, and fracktalkine (CXC3L1) (Table 1).[29,34,35]

IL6, the key cytokine in CRS, is a pleotropic cytokine that displays both anti-inflammatory and pro-inflammatory characteristics. It is mainly secreted by lymphocytes, but it might also be generated by monocytes/macrophages, dendritic cells, T-cells, adipocytes, mesenchymal cells, and osteoblasts. IL6 is assigned in many systems, such as the neutrophil migration, acute phase response, angiogenesis, B-cell differentiation, antibody generation,

Table 1
Principle cytokines associated with CRS and their effects.

cytokine	source	target and effect
IFN-γ	NK cells, Th1 cells and CTLs	Macrophage activation, Th1 cell differentiation, B cell isotype switching increases MHC expression and antigen processing to T cells
TNF-α	Macrophages, NK cells and T cells	Endothelial cell activation (inflammation), microbicidal activity in neutrophils and macrophages, synthesis of acute phase proteins in liver
IL1 β	Macrophages, DCs, fibroblasts, endothelial cells, hepatocytes	Endothelial cell activation (inflammation, coagulation), synthesis of acute phase proteins in liver
IL2	T cells	proliferation and differentiation of T cells and NK cells B cell proliferation and antibody synthesis
IL6	T cells, monocytes, macrophages, fibroblasts, and endothelial cells	Augment immune response proliferation of antibody-producing B cells Neutrophil production from the bone marrow synthesis of acute phase proteins in Liver
IL10	Th2 cells and macrophages	inhibition of the expression of IL-12 in Macrophages and DCs
IL12	Macrophages and DCs	Th1 cell differentiation IFN-γ synthesis in NK cells and T cells increasing cytotoxicity
IL8	Macrophages, epithelial cells, airway myocytes, monocytes, T lymphocytes, neutrophils, vascular endothelial cells, dermal fibroblasts, keratinocytes, hepatocytes	Lymphocyte and neutrophil chemotaxis and induction of phagocytosis (migration exocytosis; release of some mediator such as histamine), respiratory burst, Chemoattractant for endothelial cell, macrophage, mast cell and keratinocyte promotes angiogenic responses in endothelial cell, autocrine growth factor for cancer cells
Fractalkine	Monocytes, endothelial cells, macrophages, DCs, fibroblasts (by stimulation of cytokines such as TNF-α, IN-γ and IL1-β)	Membrane binding form; leukocytes adhesion Soluble form; chemoattractant for monocytes, NK cells and T lymphocytes An important receptor and surface marker for NK cells and CTL

CTL: cytotoxic T lymphocytes NK: natural killer DCs: dentritic cells.
References: [30,34,35].
Adapted from reference [30] with the permission of Yong-Min Tang.

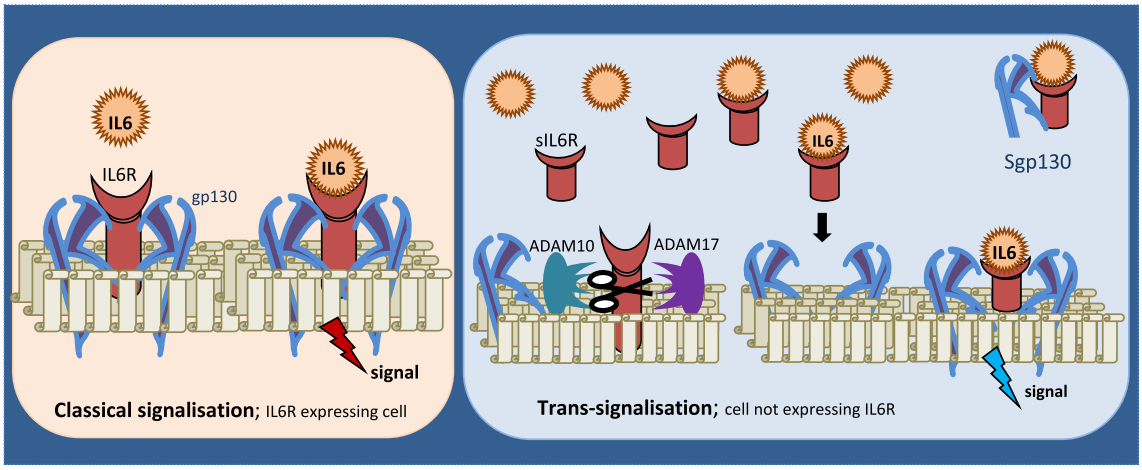

Fig. 1. IL6 signalization mechanisms: IL6 binds to the IL6 receptor (IL6R) which present with glycoprotein 130 (gp130) in the membrane and activates intracellular signaling called as 'classical signaling'. The sIL6R, soluble form of IL6R, combines with IL6 in body fluids. This complex may also bind to the cells that hold gp130 alone. So by this way is called 'trans-signaling' IL6 can affect the cells that not have IL6R. ADAM 10 and ADAM 17 metalloproteases are responsible for cleavage and consist of sIL6R. Soluble gp130 (sgp130) preferentially can binds the IL-6/sIL-6R complex to antagonize IL-6 trans-signaling.

and lipid metabolism, that either directly concern the immune system or not.[36] IL6 binds to the IL6 receptor (IL6R, CD126, gp80) presented in the membrane together with the signal transducer membrane protein gp130 (CD130). This complex activates such pathways as JAK/STAT, PI3K, and RAS/MAP kinase and starts intracellular signaling by causing gp130 to dimerize.[37] Healthy signaling requires the existence of gp130 and IL6R on the surface of the same cell. This is called as 'classical signaling' (Fig. 1). While gp130 is present on the surface of many cells, IL6R is mainly found in such immune effector cells as neutrophils, monocytes/macrophages, and lymphocytes and some non-immune cells such as hepatocytes. Thus, a limited group of cells is sensitive to IL6. IL6R can also be found in soluble form in body fluids (sIL6R), which can bind cells that hold gp130 alone on their surfaces. While the soluble receptors of TNFα and IL1 function as an antagonist, sIL6R functions as an agonist. When IL6 attaches to sIL6R and starts the signaling by binding to cells that hold gp130 alone -and not accompanied with IL6R-on their surfaces, this is called 'trans-signaling' (Fig. 1). Many cells such as embryonic stem cells, early hematopoietic progenitor cells, neural cells, and, endothelial cells are not sensitive to IL6 under normal conditions, because they do not carry IL6R. But, they do become sensitive when sIL6R is present.[38] It is considered that the anti-inflammatory activity of IL6 depends on classical signaling, while its pro-inflammatory activity depends on trans-signaling. sIL6R is mainly formed two ways. The first is through limited proteolytic cleavage from the cell membrane and the second is

transmission from spliced m-RNA.[37] A recent study demonstrated that deactivation of the sIL6R gene significantly decreased sIL6R levels and that the main source of sIL6Rs in the circulation were hepatocytes and hematopoietic cells. However, it is mainly produced by proteolytic cleavage particularly in chronic inflammations and malignancies. ADAM 17 and ADAM 10 metalloproteases (sheddase), found in the membrane, are thought to be responsible for cleaving the IL6R through proteolysis and for releasing sIL6R into the environment. Pro-inflammatory cytokines such as IL1β, TNFα and bacterial toxins cause sIL6R to increase through apoptosis by intrinsic or extrinsic factors such as DNA-damage and radiation and by activating the ADAM 17 enzyme.[39,40] Also, when IL6 level is low, it primarily binds to the IL6R bound to the membrane, resulting in classical signaling. On the contrary, when IL6 levels are high, 'trans-signaling' is also triggered activating more cells.[41] Therefore, high IL6 levels, cell membrane destruction, and apoptosis trigger the pro-inflammatory cascade causing CRS.

Destruction of target cells or activation of CART cells causes not only the release of IL6, but also cytokines such as IFNγ, TNFα, IL1β, and IL2 from lymphocytes, monocytes, and neutrophils. IFNγ, generated by T-cells and NK-cells, is a proinflammatory cytokine that plays a role in the T-cell differentiation, MHC1 induction, and B-cell isotype shift.[42] It also activates macrophages and causes the release of cytokines such as TNFα, IL1β, IL6, IL8, and IL10. This cascade contributes to the cytokine storm. At this point, IL10 fails to control although it suppresses cellular immunity.

4. Symptoms and findings

Clinical symptoms and the severity of CRS may vary significantly. It can cause fever, chills, fatigue, nausea, headache, muscle pain, dyspnea, tachycardia, hypotension, liver dysfunction, and even respiratory distress syndrome with a fatal progress potential, acute vascular leak syndrome, disseminated intravascular coagulopathy, neurotoxicity, cardiac dysfunction, renal failure, and multiple organ failure.

In general, fever does not exceed 40.0 °C and is the first and the most important symptom. The onset and duration of fever vary according to the agent. Most frequently fever onsets within hours after monoclonal antibodies. Whereas, in CAR-T cell therapy fever generally occurs within 5–7 days after infusion, late fever rarely occurs after around 2–3 weeks.[43] Fever can be mistaken for infection. Mostly, it is not possible to distinguish the two factors so empirical antibiotic therapy should be started after collecting the appropriate cultures, particularly in neutropenic patients. Sepsis, frequently encountered in cancer patients, may also be confused with CRS. Although IL6 and IL10 levels rise quite high in sepsis, no significant increase is observed in INFg levels, making it an important parameter to distinguish sepsis from CRS.[44]

Hypotension is also frequently observed. Although rapid fluid replacement is often sufficient, vasopressor support may also be required and in general it responds well to anti-cytokine treatment. One should take into account that pulmonary edema due to vascular leak syndrome may complicate the fluid replacement.

Cardiac dysfunction is generally reversible although it can appear fast and severe. Although its pathophysiology is not exactly known, clinical findings are very similar to sepsis associated cardiomyopathy or stress cardiomyopathy (Takotsubo cardiomyopathy).[45]

The pathophysiology of neurological complications of CRS is not fully understood. It is known that it is different from the classical symptoms of CRS. Its progression and severity are quite variable. It can occur together other symptoms, but it might exist solitary as well. It might also appear during the recovery period of CRS or even become evident with anti-cytokine treatment. Some say that high cytokine levels in the cerebrospinal fluid (CSF), especially the direct neurotoxic impact of IL6, are responsible. Neurological impacts of IL6 and its association with such diseases as Alzheimer, Parkinson, multiple sclerosis, schizophrenia, and depression have been reported.[46] Neural cells, which do not contain IL6R under normal conditions, may become sensitive to IL6 since sIL6R will increase in the CSF causing trans-signaling. On the contrary, neurotoxicity after tocilizumab treatment, is explained by reduced IL6 clearance resulting in a dramatic increase of IL6 levels due to inhibition of the receptor-mediated endocytosis of IL6.[41]

CRS may also be mistaken or occur together with hemophagocytic lymphohistiocytosis (HLH). HLH is a rarely encountered immune regulation disorder, which results in excessive inflammation and pathological immune activation. Besides high cytokine levels, high ferritin and triglyceride levels, fever, cytopenia, splenomegaly, and impaired coagulation are the characteristics of this syndrome. Primary HLH is a hereditary disease caused by defective cytolytic exocytosis and macrophage activation syndrome (MAS) is a form of secondary HLH caused by infection, malignancy, or autoimmune diseases. It has been shown that many patients diagnosed with MAS had heterozygote gene mutations as PRF1, MUNC13-4, STXBP2, and STX11.[47,48] CAR T cell therapies or other immunotherapies may trigger these syndromes without any mutation. MAS and CRS share the same clinical and laboratory properties suggesting a similar pathophysiology. Some consider CRS caused by T cell therapies as a part of HLH or MAS as well. The association of MAS and CRS was suggested by Maude et al., in 2014.[15] They point out the coexistence of hepatosplenomegaly, liver dysfunction, hypofibrinogenemia, coagulopathy, and severe hyperferritinemia accompanied by high fever in some patients diagnosed with CRS. Even cytokine patterns of these patients were similar to those of patients with MAS, such as high levels of IL10, IL6, and IFNγ, but also high levels of IL2R, MCP-1, and MIP1B with normal levels of IL1β, IL4, IL5, IL7, IL12, IL13, IL17, TNFα, and GM-CSF).[47,49,50] Grupp et al. also reported two cases of MAS developed together with CRS after CD19-CAR therapy.[51]

Depending on the cytokine activation renal failure may develop during the course of CRS. In addition, it should keep in mind that, tumor lysis syndrome might simulate or attribute to CRS due to the anti-tumor effect of the agent. In tumor lysis syndrome excessive cytokine levels contribute to inflammation, acute renal failure, and hypotension. However, metabolic abnormalities such as hyperphosphatemia, hyperkalemia, and hypocalcemia, which are often seen in tumor lysis syndrome, are not common in CRS.[52] A careful differential diagnosis should be made including these two syndromes and should be treated accordingly.

5. Biomarkers

Since CRS basically arises from supra-physiological levels of cytokines, in theory, cytokines may be used as a marker in predicting the course and severity of the syndrome. Most of the studies on this subject indicates that the tumor burden, high cytokine/protein levels (especially C-reactive protein (CRP), ferritin, interferon-g (IFNg), and interleukin-6 (IL-6) but also sIL2Rα, MCP1, sgp130), and severity of CRS are correlated, even though opinions on the contrary do also exist.[15,24,31,41,53–55] But the main question is whether in the early period these inflammatory markers or cytokines can anticipate the severity of CRS and can be practical as a tool to guide prophylactic or preemptive treatment. The predictive values and feasibility of these markers are controversial.

First of all, standardization and accessibility of cytokine measurement are not optimal. Also, threshold values for cytokine measurement are not yet defined. Second, in spite of the fact that CRS correlates with the cytokine level, it is not clear whether it can act as a predictor in those cancer patients, in whom cytokine levels

may already be high. Moreover, the cytokine profile of CRS may overlap with those of MAS/HLH and infection. Therefore, it may be more reasonable to calculate the increase as a ratio or the increase compared to the basal value instead of the absolute cytokine level. In fact, it seems rational to use several markers at the same time and to follow up a series of measurements rather than the level of a single cytokine. However, which cytokine(s) will be more beneficial? Additional data are needed to determine. In fact, several studies proved the role of not one but many cytokines in CRS, but still, the focus of primary treatment in severe CRS is on suppressing the IL6 signaling. An IL6 targeted therapy, tocilizumab, is used as first line immunosuppressive therapy, rather than anti-TNFα or anti-IL1 treatment. Thus benefit of broad cytokine measurements in the management of the syndrome is questionable at the moment, because staging and treatment are ultimately based on clinical parameters.

CRP, an acute phase reactant, is increased significantly during inflammation and might provide a prediction on the bioactivity of IL6. This marker might be preferred because of inexpensiveness and feasibility. Many studies demonstrated that, there is a significant correlation between CRP concentration and severity of CRS, and also response to treatment but CRP falls behind regarding the increasing time of other cytokines.[24,31,54] Besides, as CRP is also high in infection, it is not useful in discriminating these two conditions or in overlapping cases. Likewise ferritin, another acute phase reactant, will be useful to monitoring but not enough as a predictive marker. Some suggest that so high ferritin level (>20,000 ng/ml) or CRP level (>150 mg/L) could serve as a useful marker as it is not typical for infection even in heavily transfused patients. Common view is that; routine monitoring of CRP and ferritin concentration may be beneficial but more studies are needed to prove the predictive significance of these markers.

The correlations between serum IL-6, IFN-γ levels and severity of CRS were revealed as well. Moreover elevated levels of these cytokines can be detected earlier than CRP and ferritin.[24,31] But these tests are not easily available and still data are needed to determine the thresholds for risk stratification and guiding the therapy.

6. Staging and treatment

NCI-CTCAEs has defined certain criteria and grades for CRS as in many pathological conditions.[1] This system is quite basic but, most of the patients also display additional morbidities such as neutropenia, immune suppression, infection, and tumor lysis syndrome and so a more detailed clinical evaluation is needed to distinguish the causes and treat them accordingly. Subsequently, some authors revised these criteria taking previous experiences into account.[41,54] CRS grading scale published by Lee and colleagues is quite practical and useful (Table 2).[41] But it should be noted that, this scale is not include any laboratory marker. As the data about the effect of cytokine levels on prognosis increase, a new revision may be needed.

Grade 1 defines patients with mild symptoms. Interruption of treatment is not required and symptomatic treatment will be sufficient.

Grade 2 defines patients with respiratory distress, which gets well with less than 40% oxygen, hypotension responding to parenteral fluid therapy or low dosage vasopressor, and grade 2 organ toxicity. Interruption of treatment is required; however, symptomatic treatment also provides rapid response. Age and comorbidity of the patient should be taken into account before deciding to intervene with an immunosuppressive in a patient at grade 2.

Grade 3 defines patients with hypotension unresponsive to fluid and low dosage vasopressor therapy, respiratory distress that does not respond to low dosage of oxygen therapy, and grade 3 organ dysfunctions such as coagulopathy, renal and cardiac dysfunction. The symptoms persist even though infusion is interrupted and symptomatic treatment is carried out properly. These patients should be rapidly and closely monitored under intensive care conditions. Possible cardiac decompensation may be overlooked in patients not properly monitored and not evaluated by echocardiography. Anti-cytokine treatment is required to prevent permanent organ dysfunction.

Grade 4 defines patients with fatal risk. Vasopressor treatment and mechanical ventilation is necessary. These patients should rapidly receive anti-cytokine treatment.

Another scoring system SOFA (sequential organ failure assessment) has been developed for the patients in intensive care unit. This scoring system includes blood pressure, platelet count, creatinine and bilirubin levels, partial oxygen pressure, and Glasgow coma scale parameters. Increased cytokine levels in patients receiving the CAR T cell therapy were demonstrated to be associated with a high SOFA score.[55]

7. Treatment

Tocilizumab, a monoclonal IL6R antibody, inhibits both classical and trans-IL-6 signaling in the IL6 pathway via blocking IL-6 binding to both cell-associated and soluble IL-6Rs. Tocilizumab is an approved drug for the treatment of rheumatoid arthritis since 2010 and also there are many experiences in the treatment of CRS.[24,31,55–57] In 2017, Tocilizumab has been approved by FDA for the treatment of CAR T cell induced severe CRS in patients 2 years old and over. In rheumatoid arthritis recommended dosage is 4–8 mg/kg every 4 weeks in adults and 8–12 mg/kg every 2–4 weeks in children and in studies conducted on CRS doses in similar range were used. The approved dosage of tocilizumab for CRS is 12 mg/kg for patients less than 30 kg weight and 8 mg/kg for patients at or above 30 kg weight. Fever and hypotension generally ameliorates within a few hours in patients responsive to tocilizumab. However, in some patients it may be necessary to continue supportive treatment for several days. Although Tocilizumab has a very long half life (11–14 days) the common approach is to repeat the dose if enough clinical improvement is not achieved within 48 h.[41,57,58] If the patient still does not improve with persisting high IL6 levels, a high dose of tocilizumab may be considered, although no sufficient data exist on this topic.

Neurological symptoms may react differently in CRS. Sometimes, neurological symptoms may be prolonged in patients who have rapid hemodynamic response to tocilizumab. Some say that this is dependent on the direct neurotoxic impact of IL6. Both the transmission of IL6 produced at periphery and activated immune cells into the CNS are the source of increased IL6 in CNS. Tocilizumab does not pass the CSF barrier easily. After administration of tocilizumab a temporary increase of IL6 is seen as a result of the receptor blockage and inhibition of endocytosis.[41] Consequently, neurological symptoms are in fact initially exacerbated after tocilizumab or even some neurological symptoms may emerge. Therefore, tocilizumab is not an appropriate option for a CRS patient, who has grade 3 to 4 neurological findings but does not display a significant hemodynamic disturbance. In these cases steroids will probably be more beneficial.

The most frequent adverse effects reported in patients with rheumatoid arthritis are transaminitis, thrombocytopenia, and hypercholesterolemia. Neutropenia was also seldom reported resolving after discontinuing. In chronic use there might be an increase of the infection risk. Severe adverse effects (grade 3 or greater) are extremely rare.[59]

Table 2
Revised grading system and management of CRS.

	Symptoms	Treatment
Grade 1	Mild constitutional symptoms such as fever, nausea, fatigue, headache, myalgia, malaise	Not require interruption of therapy Symptomatic treatment ± empiric treatment of concurrent bacterial infections
Grade 2	Symptoms require moderate intervention • Hypoxia with oxygen requirement less than 40% • Hypotension responds to fluids or low dose vasopressor* • Grade 2 organ toxicity**	Interruption of therapy is required, rapid response to symptomatic treatment Immunosuppressive treatment is optional according to the comorbidities or age
Grade 3	Symptoms require aggressive intervention • Hypoxia with oxygen requirement more than 40% • Hypotension not responds to low dose vasopressor* (requires high dose or multiple vasopressors) • Grade 3 organ toxicity -such as coagulopathy, renal dysfunction, cardiac dysfunction- or grade 4 transaminitis**	Prolonged duration of symptoms despite symptomatic treatment and interruption of therapy. Monitorization in ICU aggressive intervention with immunosuppressive treatment (tocilizumab ± corticosteroids) is required.
Grade 4	Life-threatening symptoms and toxic condition • Grade 4 organ toxicity** (excluding transaminitis)	Ventilator support and vasopressors are required Rapid intervention with immunosuppressive treatment (tocilizumab ± corticosteroids)
Grade 5	Death	

*low dose vasopressors.
Norepinefrin ≤20 mcg/kg/dak.
Dopamin ≤10 mcg/kg/dak.
Epinefrin ≤10mcg/kg/dak.
**Grades of toxicities are dictated by CTCAE v5.0.
References: [1,41].
Adapted from reference [41] with the permission of Crystal L. Mackall

Steroids are frequently used to suppress an extremely inflammatory response in sepsis and to diminish the cytokine release caused by HLH/MAS with success. Also steroids appear to be successful in CRS after administration of a moAb.[41] Being familiar and available are the advantages of steroids but their efficacy in the CRS management is slower compared to that of tocilizumab. In addition, since they have undesired effects on the T-cells, and potentially could decrease the antitumor effect of the CAR T cells, corticosteroid treatment should be considered in second line treatment for patients refractory to tocilizumab. Although depending on patient characteristics, the generally preferred steroid dosage is 2 mg/kg/day methylprednisolone. Dexamethasone may be preferred in patients with dominant neurological symptoms since it penetrates the blood brain barrier more effectively. It should also be pointed out that a relative corticosteroid deficiency may arise because of the suppression of the hypothalamic-pituitary axis when CRS develops in patients, who are frequently administered corticosteroids in the treatment process as in the case of B-ALL, and in such a case a stress dosage of corticosteroid replacement will be appropriate.

Targeted immunosuppressive therapies for other cytokines such as anti-TNFα moAb infliximab, the TNFα inhibitor etanercept, and the IL1R inhibitor anakinra are alternatives that may be considered in the management of CRS. Although, these agents and also Cyclosporine are known to be efficient on HLH/MAS which have similar pathophysiological features with CRS, for the present, there are not enough experiences and data on CRS. The anti-CD25 moAb daclizumab has gained attention after studies showed an increase of the soluble IL2 receptor CD25 in patients with HLH after the CAR-T cell therapy.[60] For patients who become critically ill and do not respond to IL6 directed therapy, targeting IL1, IFNγ, TNFα or sIL2Rα could ameliorate the symptoms. But it should keep in mind; these therapies would likely decrease antitumor efficiency of the CAR T cells.

Another moAb that targets IL6, siltuximab, has approval in the treatment of HHV-8 and HIV negative Multicentric Castelman's Disease and has been the potential to be effective in the treatment of CRS. Also there are some data on the efficacy of siltuximab in some inflammatory and malignant diseases such as juvenile idiopathic arthritis, multiple myeloma and prostate cancer, but it has not been studied as a first-line therapy in CRS. There are a few experience in patients unresponsive to both tocilizumab and corticosteroid treatment but future studies are needed.[43,61–63]

The 'soluble gp130' is a particularly promising molecule with growing data on many diseases such as osteoarthritis, chronic colitis, atherosclerosis, and cancer.[64] Soluble gp130 binds the IL6/sIL6R within the extracellular matrix and prevents activation in the membrane, in this way excessive inflammation is prevented via inhibition of trans-signaling without affecting classical signaling.[37] This may be an advantage when compared with tocilizumab. More data on the use of this molecule in CRS treatment are needed.

Another topic is whether prophylactic or preemptive treatment with IL6 directed therapy would be of benefit, or not. It is not clear whether cytokine release in some degree is necessary for antitumor effect and intervening with them might cause undesired effect. In fact, thus far, treatment with tocilizumab at the time of grade 3–4 CRS does not seem to adversely impact the CAR T cells or disease outcomes. And also, given the rapid, dramatic clinical response and lack of apparent side effects, tocilizumab has become a standard therapy in CRS.[24,31,55–57] Gardner et al. reported the experience with preemptive tocilizumab in 20 patients with CRS.[55] They revealed that early intervention with tocilizumab -just after the onset of clinical symptoms-decreases the severity of CRS without affecting the rates of remission, the engraftment and expansion of $CAR^{+}T$ cells, and the rates of neurotoxicity. Likewise Maude et al. reported that prophylactic treatment with tocilizumab did not affect the success of the CAR T cell therapy.[56] Although there is no approval on prophylactic or preemptive treatment, a benefit to using tocilizumab to prevent CRS particularly in patients with high risk warrants specific trials.

Prophylactic treatment with steroid may be considered to prevent CRS or at least to diminish the symptoms. But the potential damage of steroids on the T lymphocyte is still a reason to restrict the use of steroids in course of cellular therapies. Some studies, however, reported that steroid therapy did not suppress T cell activation and not affect the response rates; there are some opposing reports.[28,54,65,66] Long-term efficacy is remains unknown. Given these concerns, common view is that, the prophylactic use of corticosteroids should be avoided, even in premedication for blood

transfusion.[16,56,67]

Unlike CART cell induced CRS, corticosteroids are recommended as prophylaxis and first line treatment in the management of blinatumomab induced CRS. Studies revealed that inhibition of cytokines by corticosteroids do not decrease the efficiency of blinatumomab on activation of T cells.[65] Indeed, since premedication with dexamethasone has been used routinely, in current studies on blinatumomab CRS was reported less frequently.

The field of immunortherapies including moAbs and cellular therapies is rapidly evolving. These agents have a great and impressive potential in 'fighting with cancer', but effective utilization of this new therapeutics requires a proper management of toxicities. Here some of the studies conducted on CRS and developments that have been achieved with regard to control and prevention of this syndrome are compiled. However many questions remain. What drives the inflammatory response from normal to excessive such as in this syndrome? Why is the inflammation more severe in some patients? Can we predict who will develop severe CRS? Can measurement and monitoring cytokine levels before treatment and studying gene polymorphism be beneficial? Future studies will hopefully shed a light into these questions.

References

1. NCI-CTC, Common Terminology Criteria for Adverse Events (CTCAE) v5.0 Published: 27 november 2017 https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf Accessed 04 June 2018.
2. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. *Am J Emerg Med*. 2008;26:711–715.
3. von Wussow P, Platsoucas CD, Wiranowska-Stewart M, Stewart 2nd WE. Human gamma interferon production by leukocytes induced with monoclonal antibodies recognizing T cells. *J Immunol*. 1981;127:1197–1200.
4. Dillman RO, Shawler DL, Sobol RE, et al. Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukemia. *Blood*. 1982;59:1036–1045.
5. Foon KA, Schroff RW, Bunn PA, et al. Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia. *Blood*. 1984;64:1085–1093.
6. Wing MG, Moreau T, Greenwood J, et al. Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgRIII) and CD11a/CD18 (LFA-1) on NK cells. *J Clin Invest*. 1996;98:2819–2826.
7. Adams PS, Shapiro R, Hilmi IA. Postoperative cardiac tamponade after kidney transplantation: a possible consequence of alemtuzumab-induced cytokine release syndrome. *Transplantation*. 2013;95:e18–19.
8. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412N. *Engl J Med*. 2006;355:1018–1028.
9. Shan P, Ledbetter JA, Press OW. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. *Blood*. 1998;91:1644.
10. Byrd JC, Waselenko JK, Maneatis TJ, et al. Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. *J Clin Oncol*. 1999;17:791–795.
11. Kasi PM, Tawbi HA, Oddis CV, Kulkarni HS. Clinical review: serious adverse events associated with the use of rituximab - a critical care perspective. *Crit Care*. 2012;16:231.
12. Makino K, Nakata J, Kawachi S, Hayashi T, Nakajima A, Yokoyama M. Treatment strategy for reducing the risk of rituximab-induced cytokine release syndrome in patients with intravascular large B-cell lymphoma: a case report and review of the literatüre. *J Med Case Rep*. 2013;30:280.
13. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine release syndrome in patients with B-Cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (Rituximab, IDEC-C2B8). *Blood*. 1999;94:2217–2224.
14. Hannawa IS, Bestul DJ. Rituximab tolerability when given before or after CHOP. *J Oncol Pharm Pract*. 2011;17:381–386.
15. Maude SL, Barrett D, Teachey DT, Grupp SA. Managing cytokine relase syndrome associated with novel T cell engaging therapies. *Cancer J*. 2014;20:119–122.
16. Nellan A, Lee DW. Paving the road ahead for CD19 CAR T-cell therapy. *Curr Opin Hematol*. 2015;22:516–520.
17. Nagorsen D, Kufer P, Baeuerle PA, Bargou R. Blinatumomab: a historical perspective. *Pharmacol Ther*. 2012 Dec;136:334–342.
18. Viardot A, Goebeler MA, Hess G, Neumann S, Pfreundschuh M, Adrian N. Phase 2 study of the bispecific T-cell engager (BiTE) antibody Blinatumomab in relapsed/refractory diffuse large B-cell lymphoma. *Blood*. 2016;127:1410–1416.
19. Buechner J, Grupp SA, Maude SL, et al. Global registration trial of efficacy and safety of CTL019 in pediatric andyoung adult patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL): update to the interim analysis. *Clin Lymphoma, Myeloma & Leukemia*. 2017;17:S263–S264.
20. Hay KA, Hanafi LA, Li D, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 Chimeric antigen receptor-modified T cell therapy. *Blood*. 2017;130:2295–2306.
21. Schuster SJ, Bishop MR, Tam C, et al. Global pivotal phase 2 trial of the Cd19-targeted therapy Ctl019 in adult patients with relapsed or refractory (R/R) diffuse large B-cell lymphoma (Dlbcl) -an interim analysis. *Hematol Oncol*. 2017;35:27.
22. Gardner RA, Finney O, Annesley C, et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood*. 2017;129:3322–3331.
23. Abramson JS, Palomba ML, Gordon LI, et al. High durable CR rates in relapsed/refractory (R/R) aggressive B-NHL treated with the CD19-directed CAR T cell product JCAR017 (TRANSCEND NHL 001): defined composition allows for dose-finding and definition of pivotal cohort. *Blood*. 2017;130:581.
24. Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov*. 2016 Jun;6:664–679.
25. Grupp SA, Laetsch TW, Buechner J, Bittencourt H, Maude SL. Analysis of a global registration trial of the efficacy and safety of CTL019 in pediatric and young adults with relapsed/refractory acute lymphoblastic leukemia (ALL). *Blood*. 2016;128:221.
26. Maude SL, Laetsch W, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439–448.
27. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-t cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126:2123–2138.
28. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377:2531–2544.
29. Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor Tcells. *Front Pharmacol*. 2014;28:235.
30. Xu XJ, Tang YM. Cytokine release syndrome in cancer immunotherapy with chimeric antigen receptor engineered T cells. *Cancer Lett*. 2014;343:172–178.
31. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126:2123–2138.
32. Dahmer MK, Randolph A, Vitali S, Quasney MW. Genetic polymorphisms in sepsis. *Pediatr Crit Care Med*. 2005;6:61–73.
33. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther*. 2010;18:843–851.
34. Jones BA, Beamer M, Ahmed S. Fractalkine/CX3CL1: a potential new target for inflammatory diseases. *Mol Interv*. 2010;10:263–270.
35. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *J Leukoc Biol*. 1994;56:559–564.
36. Mihara M, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci*. 2012;122:143–159.
37. Rose-John S, Scheller J, Elson G, Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J Leukoc Biol*. 2006;80:227–236.
38. Jones SA, Richards PJ, Scheller J, Rose-John S. IL-6 transsignaling: the in vivo consequences. *J Interferon Cytokine Res*. 2005;25:241–253.
39. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta*. 2011;1813:878–888.
40. Chalaris A, Gewiese J, Paliga K, et al. ADAM17-mediated shedding of the IL6R induces cleavage of the membrane stub by gamma-secretase. *Biochim Biophys Acta*. 2010;1803:234–245.
41. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124:188–195.
42. Saha B, Jyothi Prasanna S, Chandrasekar B, Nandi D. Gene modulation and immunoregulatory roles of interferon gamma. *Cytokine*. 2010;50:1–14.
43. Teachey DT, Bishop MR, Maloney DG. Grupp SA Toxicity management after chimeric antigen receptor T cell therapy: one size does not fit'ALL'. *Nat Rev Clin Oncol*. 2018 Apr;15:218.
44. Xu XJ, Tang YM, Liao C, et al. Inflammatory cytokine measurement quickly discriminates gram-negative from gram-positive bacteremia in pediatric hematology/oncology patients with septic shock. *Intensive Care Med*. 2013;39:319–326.
45. Singh K, Carson K, Shah R, et al. Meta-analysis of clinical correlates of acute mortality in Takotsubo cardiomyopathy. *Am J Cardiol*. 2014;113:1420–1428.
46. Spooren A, Kolmus K, Laureys G, et al. Interleukin-6, a mental cytokine. *Brain Res Brain Res Rev*. 2011;67:157–183.
47. Risma K, Jordan MB. Hemophagocytic lymphohistiocytosis: updates and evolving concepts. *Curr Opin Pediatr*. 2012;24:9–15.
48. Zhang K, Jordan MB, Marsh RA, et al. Hypomorphic mutations in PRF1,

MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. *Blood*. 2011;118:5794–5798.
49. Tang Y, Xu X, Song H, et al. Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome. *Br J Haematol*. 2008;143:84–91.
50. Teachey DT, Rheingold SR, Maude SL, et al. Cytokine release syndrome after blinatumomab treatment related to abnormal macrophage activation and ameliorated with cytokine directed therapy. *Blood*. 2013;121:5154–5157.
51. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368:1509–1518.
52. Cairo MS, Bishop M. Tumour lysis syndrome: new therapeutic strategies and classification. *Br J Haematol*. 2004;127:3–11.
53. Frey N. The what, when and how of CAR T cell therapy for ALL. *Best Pract Res Clin Haematol*. 2017;30:275–281.
54. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6:224–225.
55. Kochenderfer JN, Dudley ME, Feldman SA, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor transduced T cells. *Blood*. 2012;119:2709–2720.
56. Maude SL, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia. *Blood*. 2015;125:4017–4023.
57. Fitzgerald JC, Weiss SL, Maude SL, et al. Cytokine release syndrome after chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia. *Crit Care Med*. 2017:e124–e131.
58. U.S. Food & Drug Administration. Official website. www.accessdata.fda.gov/drugsatfda_docs/label/2017/125276s114lbl. Accessed June 4, 2018.
59. Navarro G, Taroumian S, Barroso N, Duan L, Furst D. Tocilizumab in rheumatoid arthritis: a meta-analysis of efficacy and selected clinical conundrums. *Semin Arthritis Rheum*. 2014;43:458–469.
60. Tomaske M, Amon O, Bosk A, Handgretinger R, Schneider EM, Niethammer D. Alpha-CD25 antibody treatment in a child with hemophagocytic lymphohistiocytosis. *Med Pediatr Oncol*. 2002;38:141–142.
61. Chen F, Teachey DT, Pequignot E, et al. Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy. *J Immunol Methods*. 2016;434:1–8.
62. Leurs A, Launay D, Terriou L, Hatron PY, Quartier P, Hachulla E. Remission of refractory systemic-onset juvenile idiopathic arthritis after treatment with siltuximab. *J Clin Rheumatol*. 2018. https://doi.org/10.1097/RHU.0000000000000716. PMID: 29485546 February 27, 2018.
63. van Rhee F, Wong RS, Munshi N, et al. Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2014;15:966–974.
64. Jostock T, Müllberg J, Ozbek S, AtreyaR, Blinn G, Voltz N. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur J Biochem*. 2001;268:160–167.
65. Brandl C, Haas C, d'Argouges S, et al. The effect of dexamethasone on polyclonal T cell activation and redirected target cell lysis as induced by a CD19/CD3-bispecific single-chain antibody construct. *Cancer Immunol Immunother*. 2007;56:1551–1563.
66. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med*. 2011;365:725–733.
67. Gardner R, Leger KJ, Annesley CE, Summers C, Rivers J, Gust J. Decreased rates of severe CRS seen with early intervention strategies for CD19 CAR-T cell toxicity management. *Blood*. 2016;128:586.