

How I Treat– Current Concepts in the Diagnosis and Management of Cytokine Release Syndrome

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ABSTRACT

As immune based therapies for cancer become potent, more effective and more widely available, optimal management of their unique toxicities becomes increasingly important. Cytokine release syndrome (CRS) is a potentially life threatening toxicity that has been observed following administration of natural and bi-specific antibodies and more recently, following adoptive T cell therapies for cancer. CRS is associated with elevated circulating levels of several cytokines including IL-6 and IFN γ , and uncontrolled studies demonstrate that immunosuppression using tocilizumab, an anti-IL6R antibody, with or without corticosteroids, can reverse the syndrome. However because early and aggressive immunosuppression could limit the efficacy of the immunotherapy, current approaches seek to limit administration of immunosuppressive therapy to patients at risk for life threatening consequences of the syndrome. This report presents a novel system to grade the severity of CRS in individual patients, and a treatment algorithm for management of CRS based upon severity. The goal of our approach is to maximize the chance for therapeutic benefit from the immunotherapy while minimizing the risk for life threatening complications of CRS.

Introduction

Cancer immunotherapy seeks to harness the power of the immune system to eradicate malignant tissues. After decades of research, numerous cancer immunotherapies have shown definitive clinical efficacy, spanning GvL to eradicate leukemia following HSCT¹, mAbs to improve survival for patients with B cell lymphomas and HER-2 expressing breast cancer^{2,3}, and a therapeutic cancer vaccine for hormone refractory prostate cancer⁴. Recently, mAbs that block key checkpoints on T cells have improved survival in metastatic melanoma and induced antitumor effects in other cancers^{5,6}, bi-specific mAbs have mediated impressive responses in B cell acute lymphoblastic leukemia⁷ (ALL) and dramatic antitumor effects have been observed using adoptive T cell immunotherapy, increasingly employing genetic engineering to create tumor antigen specific T cells⁸⁻¹⁴.

Risks associated with cancer immunotherapy can be broadly classified into autoimmune toxicity and cytokine associated toxicity. Autoimmune toxicity, so-called “on target, off-tumor toxicity”, results from antigen-specific attack on host tissues when the targeted tumor associated antigen is expressed on non-malignant tissue. Autoimmune toxicity occurs not uncommonly after treatment with checkpoint inhibitors^{5,6,15} and has resulted in fatal toxicities after infusion of genetically engineered T cells targeting MAGE-A3¹⁶⁻¹⁸. Cytokine associated toxicity, also known as cytokine release syndrome (CRS), is a non-antigen specific toxicity that occurs as a result of high-level immune activation. The magnitude of immune activation typically required to mediate clinical benefit using modern immunotherapies exceeds levels of immune activation that occurring in more natural settings, and as immune based therapies have become more potent, this syndrome is becoming increasingly recognized. This report reviews current concepts regarding the pathophysiology of CRS, describes the clinical manifestations of the syndrome, and provides

a grading system to assess severity and guide treatment of CRS in the context of emerging immune based therapies. Institutional IRB protocols approved all of the studies that patients discussed in this manuscript were enrolled on, and studies were conducted in accordance with the Declaration of Helsinki.

Case 1: Grade 1 CRS after CAR T cell Therapy for Leukemia

An 11-year-old with primary refractory pre-B cell ALL failed to obtain remission after 3 cycles of intensive chemotherapy and was referred for CD19-CAR therapy. At the time of enrollment, 58% of her marrow mononuclear cells were lymphoblasts. She received fludarabine and cyclophosphamide for lymphodepletion on D-4 through -2, then 1×10^6 CD19 CAR T cells/kg on Day 0. She developed fever on Day 1 that peaked at 40.7°C associated with rigors, which persisted for five days. No source of infection was identified and she remained hemodynamically stable with no evidence for neurologic dysfunction. Plasma IL-6 levels peaked at 222 pg/mL (approx. 30 fold over baseline). Assessment on Day 28 revealed minimal residual disease negative complete remission. She subsequently underwent a matched sibling HSCT and remains disease free 16 months later.

Case 2: Grade 3 CRS after Genetically Unmodified Cytotoxic T cells (CTL) Therapy for EBV-associated Lymphoma

A 19-year-old female with relapsed EBV-negative Hodgkin Lymphoma received a 9/10 HLA DRB1 mismatched unrelated HSCT following conditioning with 600 cGy TBI, fludarabine and

alemtuzumab 1H. Rapidly progressive EBV-positive post-transplant lymphoproliferative disease (EBV-PTLD) developed 3 months after transplant with elevated EBV DNA ($>100,000$ copies/ 10^6 cells) and extensive nodal involvement on PET. Biopsy confirmed EBV-PTLD. She experienced only a transient response following 2 doses of Rituximab. A donor-derived, peptide rapidly-generated multispecific (EBV, adenovirus, BK virus, and HHV6) CTL line was generated and 5×10^6 cells/ m^2 were infused on a patient specific IND. EBV DNA levels fell to normal levels with clinical and radiological improvement within two weeks after infusion. Shortly afterward, she developed fever, rash, tachycardia and hypotension with elevated IL-5, IL-13, IL-10, IL-6 and IFN γ (range 5-69 fold over baseline), associated with at least 10,000 fold expansion of EBV (EBNA1, LMP2 and BZLF1) specific CTLs in the peripheral blood. She had no evidence of tumor lysis. She was treated with dopamine and norepinephrine, etanercept and methylprednisolone (1mg/kg/day x2). Symptoms resolved within hours and vasopressor support was discontinued. Cytokine levels returned to baseline within 3 weeks. Nine months after this CRS her EBV lymphoma remains in remission.

Case 3: Grade 3 CRS after CAR T cell Therapy for Leukemia

An 11 year-old female relapsed 6 months after HSCT for B-ALL and enrolled on a clinical trial of CD19-CAR therapy. She received cyclophosphamide and fludarabine for lymphodepletion, on Day -1 her marrow showed 51% blasts, and on Day 0, 2×10^7 CD19-CAR cells were administered. On Day 1 she was admitted to the hospital with fever. Blood cultures grew *Streptococcus mitis*, and she became afebrile on vancomycin. On Day 4 she developed a new fever with headache and nausea. On Day 5 she developed tachycardia, hypotension (70/40), and

a new oxygen requirement. She received two fluid boluses and was transferred to the PICU where she received dopamine and norepinephrine. She was treated with 8mg/kg tocilizumab and within hours became afebrile, no longer required supplemental oxygen, and pressors were weaned to minimal levels. On Day 7 she became febrile again and experienced intermittent hypotension requiring continued pressor support. Ferritin (130,000 ng/ml) and CRP (21.5 mg/L) peaked on Day 7. On Day 9 she developed encephalopathy, with near aphasia, disorientation, and lethargy, which improved rapidly over the next 2 days without further immunosuppressive therapy. Pressors were discontinued on Day 10 and encephalopathy resolved completely by Day 13. She was discharged home on D15 and her marrow demonstrated a complete remission on Day 28.

Pathophysiology

CRS manifests clinically when large numbers of lymphocytes (B cells, T cells and/or NK cells) and/or myeloid cells (macrophages, dendritic cells and monocytes) become activated and release inflammatory cytokines. CRS has classically been associated with therapeutic mAb infusions, most notably anti-CD3 (OKT3), anti-CD52 (Alemtuzumab)¹⁹, anti-CD20 (Rituximab) and the CD28 super-agonist, TGN1412²⁰. In these settings, symptom onset typically occurs within minutes to hours after the infusion begins²⁰⁻²². CRS has also recently been reported following administration of bi-specific antibodies for leukemia²³, infusion of haploidentical mononuclear cells to patients with refractory leukemia²⁴ and adoptive immunotherapies for cancer, most notably T cells engineered to express CARs.^{8,25-27}

Severe CRS induced in six healthy subjects who received TGN1412 provides valuable insights into the pathophysiology of this syndrome without the background of malignant disease.²⁰ All subjects received a single dose of TGN1412 on the same day, within minutes of one another. Symptoms began approximately one hour after the infusion, and coincided with peak cytokine levels. TNF α rose first, followed by IFN γ , IL-1 β , IL-2, IL-6, IL-8 and IL-10. Within 24 hours, all subjects required transfer to an ICU for management of respiratory distress and renal dysfunction that ultimately required dialysis. Following symptom onset on Day 1, all subjects were treated with corticosteroids and within 2-3 days cytokines returned to normal levels. Two of the subjects experienced prolonged organ dysfunction well beyond the return of cytokine levels to baseline.

Timing of symptom onset and CRS severity depends on the inducing agent and the magnitude of immune cell activation. CRS following rituximab for CD20⁺ malignancies typically occurs within minutes to hours, and patients with $> 50 \times 10^9/L$ circulating lymphocytes have increased rates of CRS symptoms²². In recent reports of CRS following adoptive T cell therapy for cancer,^{10,14,28,29} the incidence and severity of the syndrome also appears greater when patients have large tumor burdens, presumably because this leads higher levels of T cell activation. In contrast, symptom onset typically occurs days (Case #1 and #3) to occasionally weeks (Case #2) after the T cell infusion, coinciding with maximal *in vivo* T cell expansion. Like CRS associated with mAb therapy, CRS associated with adoptive T cell therapies has been consistently associated with elevated IFN γ , IL-6 and TNF α levels, and increases in IL-2, GM-CSF, IL-10, IL-8, IL-5, and fractalkine have also been reported^{8,10,26,29}. A clear cell dose:response relationship for CRS related to adoptive T cell therapies has been difficult to define, but very high doses of T cells may result in earlier onset, since one case report of a fatal

event following infusion of 10^{10} CAR modified T cells bore hallmarks of CRS and had rapid onset, within minutes of infusion²⁵.

Emerging evidence implicates IL-6 as a central mediator of toxicity in CRS. IL-6 is a pleiotropic cytokine with anti-inflammatory and pro-inflammatory properties. IL-6 signaling, illustrated in Figure 1, requires binding to cell-associated gp130 (CD130), which is broadly expressed, and IL-6R (CD126)³⁰. IL-6R is cell associated on macrophages, neutrophils, hepatocytes and some T cells and mediates *classic-signaling*, which predominates when IL-6 levels are low. However when IL-6 levels are elevated, soluble IL-6R can also initiate *trans-signaling*, which occurs on a much wider array of cells³¹. Current models hold that anti-inflammatory properties of IL-6 are likely mediated via classic signaling whereas pro-inflammatory responses occur as a result of trans-signaling. High levels of IL-6, present in the context of CRS, likely initiates a proinflammatory IL-6 mediated signaling cascade.

Clinical Symptomatology

Symptomatology and severity associated with CRS varies greatly (Table 1), and management can be complicated by intercurrent conditions in these patients. Fever is a hallmark and many features of CRS mimic infection. It is not uncommon for patients to experience temperatures exceeding 40.0°C. Hence, infection must be considered in all patients presenting with CRS symptoms with appropriate cultures obtained and empiric antibiotic therapy initiated. This is of even greater importance when patients are neutropenic. Indeed, one death reported following anti-CD19 CAR based T cell therapy occurred coincident with a syndrome resembling

CRS, wherein the patient had infection predating the T cell infusion that may have contributed to the fatal outcome²⁷.

Potentially life-threatening complications of CRS include cardiac dysfunction, adult respiratory distress syndrome, neurologic toxicity, renal and/or hepatic failure and disseminated intravascular coagulation. Of particular concern is cardiac dysfunction, which can be rapid onset and severe, but is typically reversible. The pathophysiology of acute cardiac toxicity in the setting of CRS is not clear, but resembles cardiomyopathy associated with sepsis³² and stress cardiomyopathy, also called Takotsubo Cardiomyopathy³³. Neurologic symptoms occurring in the context of CRS are varied (Table 1) and may occur coincident with other symptoms of CRS, or may arise when the other symptoms of CRS are resolving (Case #3). Magnetic resonance imaging often reveals no abnormalities, although in one case, we noted an abnormality in the splenium, consistent with Mild Encephalopathy with Reversible Splenial Lesion Syndrome (MERS), a syndrome associated with infection and reversible neurologic symptoms³⁴. Given the emerging understanding of a central role for IL-6 in CRS, and the evidence that IL-6 can directly mediate neurotoxicity^{35,36}, it is of interest that MERS has also been reported to be associated with elevated IL-6 levels in the cerebrospinal fluid³⁷.

CRS may also be associated with findings of macrophage activation syndrome (MAS)/hemophagocytic lymphohistiocytosis (HLH) and the physiology of the syndromes may have some overlap.³⁸ Indeed, one patient who developed severe CRS associated with HLH following CD19-CAR therapy for ALL was found to carry a mutation in the perforin gene, which predisposes to HLH⁸. This raises the prospect that host factors may play an important role in predisposing individuals to severe CRS in the setting of immune based therapies and more studies are needed to determine whether genetic predisposition contributes to this syndrome.

Tumor lysis syndrome may also occur coincident with CRS, since massive immune cell activation and expansion correlates with antitumor efficacy. When this occurs, concomitant therapies for appropriate management of tumor lysis are essential for optimal clinical outcome.

Biomarkers

Because CRS occurs as direct result of supraphysiologic levels of inflammatory cytokines, it stands to reason that circulating cytokine levels could serve as biomarkers to diagnose and potentially quantify syndrome severity. However, several issues limit the effectiveness of this approach at the current time. First, using a biomarker as a basis for clinical management requires Clinical Laboratory Improvement Amendments (CLIA) certified assays. Such assays are not readily available to measure cytokines in most hospitals at the current time. Second, while scientific and clinical evidence implicates a relationship between inflammatory cytokine levels and CRS severity²⁶, it remains unclear whether severity in an individual patient can be predicted based upon cytokine levels. This is particularly challenging in patients with cancer, wherein baseline inflammatory cytokine levels can be very high due to their underlying disease. For this reason, fold-increases or net-increases or rate of change in cytokine levels may provide better correlates of CRS severity than absolute cytokine levels²⁶. Third, diagnostic utility could require a profile of several different cytokines, rather than changes in only one level. Finally, as discussed below, first line immunosuppressive therapy for severe and life threatening cytokine release syndrome is currently directed at preventing IL-6 signaling, despite the recognition that CRS is associated with elevations of several inflammatory cytokines. While future studies may identify settings with greater or lesser dependence on the IL-6 axis, at the current time, based upon clinical experience, we recommend use of tocilizumab as first line immunosuppressive

therapy, regardless of whether an individual patient demonstrates higher levels of TNF α or IL-1 compared to IL-6. Therefore, we conclude that real-time analysis of a broad panel of cytokines will not significantly impact management of individual patients with CRS at the current time and rather recommend that treatment decisions be based on clinical parameters.

C-reactive protein (CRP) is an acute phase reactant produced by the liver largely in response to IL-6 and CRP levels serve as a reliable surrogate for IL-6 bioactivity^{39,40}. During CRS, CRP can increase by several logs and this assay is rapid, cheap and readily available in most hospitals. In some series, peak CRP levels as well as fold-change in CRP, have identified patients at risk for severe cytokine release syndrome²⁶. In addition, we have found that declining CRP levels, while potentially lagging behind declines in cytokine levels by 1-2 days, may be useful to identify the peak of the syndrome in an individual patient. For these reasons, routine monitoring of CRP levels during the time of CRS symptomatology may have clinical utility. It is important to emphasize however that CRP levels also are elevated during infection and cannot be used to distinguish between infection-associated and non-infectious inflammation.⁴¹. We have also noted extreme elevations of ferritin in many patients with CRS after CAR T cell infusion, which further supports a link between CRS and MAS/HLH, but in our experience, ferritin has not demonstrated utility in predicting CRS severity.

Grading System and Treatment Algorithm

The National Cancer Institute Common Terminology Criteria for Adverse Events contains a grading system that was designed for CRS associated with antibody therapeutics (supplemental Table 1). We have modified this grading system to define mild, moderate, severe

and life threatening CRS regardless of the inciting agent and to guide treatment recommendations (Table 2). Because many patients with CRS have overlapping symptomatology due to fever and neutropenia, infection, tumor lysis syndrome or other medical complications it is essential that attribution be carefully considered. This is particularly important given that the treatment algorithm relies heavily on the grading schema and incorporates immunosuppression. One could imagine for example, a patient with hypotension related to sepsis wherein intervention with tocilizumab or corticosteroids (see below) would not be indicated. Hence, accurate application of this grading system requires clinical judgment to confirm that the symptoms are most likely due to CRS rather than another medical condition.

The clinical symptomatology comprising CRS is indicator of response in the setting of immune based therapies. It remains unclear to what extent the cytokines mediating the symptomatology are required for antitumor effects. Hence, the goal of management is not to extinguish all evidence of CRS, but to prevent life threatening toxicity while maximizing the potential for antitumor effects. For this reason, we recommend symptomatic treatment of Grade 1 CRS (Figure 2, Case #1). We have defined CRS as Grade 2 when the patient develops hypotension responsive to fluids or one low dose vasopressor, or mild respiratory symptoms responsive to low flow oxygen (<40% FiO₂) or Grade 2 organ toxicity. Because hypotension is a major driver of severity grading, it is imperative that a clear baseline blood pressure be established prior to initiation of therapy that could induce CRS. As shown in Figure 2, the decision to intervene with immunosuppressive agents for patients with Grade 2 CRS is influenced by the degree to which the patient is judged to be able to tolerate the altered hemodynamics and organ stresses associated with the syndrome. In older patients and patients

with significant co-morbidities, depending upon clinical judgment, it may be appropriate to intervene with immunosuppression in patients with Grade 2 CRS.

Patients in whom fluid therapy and one low dose vasopressor are not sufficient to reverse hypotension are classified as severe or Grade 3 CRS. Similarly, patients who require more than low flow oxygen or show evidence for Grade 3 organ toxicity, including but not limited to coagulopathy, renal, or cardiac dysfunction should be considered Grade 3. Patients with Grade 3 CRS need to be monitored very closely, likely in an intensive care unit with 1:1 nursing care. Importantly, in patients with Grade 2 or Grade 3 CRS, careful attention should be paid to cardiac function as cardiac decompensation may occur and may not be readily evident without careful monitoring. Frequent echocardiographic monitoring may be indicated in patients in whom there is a concern of cardiac dysfunction. All patients with Grade 3 CRS should be treated with immunosuppressive agents because of the risk for progression and the potential for irreversible organ dysfunction, which is the goal of preventing progression to Grade 4. Grade 4 CRS occurs when patients experience toxicity that is immediately life-threatening, including a need for mechanical ventilation or Grade 4 organ toxicity. We recommend that all patients with Grade 4 CRS be treated with immunosuppressive agents in an attempt to suppress the inflammatory cascade and to prevent irreversible organ dysfunction.

Tocilizumab

Tocilizumab is a humanized, IgG1 κ anti-human IL6R mAb approved for treatment of rheumatoid arthritis⁴², juvenile idiopathic arthritis⁴³ and polyarticular juvenile rheumatoid arthritis. It also has been reported to have activity in Castleman disease⁴⁴, Crohn's disease⁴⁵ and

steroid refractory chronic GVHD.⁴⁶ Tocilizumab prevents IL-6 binding to both cell associated and soluble IL-6 receptors and therefore inhibits both classical and trans-IL-6 signaling (Figure 1). Emerging clinical experience at several institutions has concluded that tocilizumab is an effective treatment for severe or life-threatening CRS^{8,22,24}. The dose of tocilizumab approved for adults with rheumatoid arthritis is 4-8 mg/kg every 4 weeks and the pediatric recommended dose is 8-12 mg/kg every 2-4 weeks. Using these recommendations as a guide, we typically administer tocilizumab over 1 hour at a dose of 4 mg/kg in adults and 8 mg/kg in children, with an option to repeat the dose if clinical improvement does not occur within 24-48 hours.

Side effects attributed to tocilizumab in rheumatologic disease, where the drug is given chronically, include transaminitis, thrombocytopenia, elevated cholesterol and low-density lipoproteins⁴². Neutropenia has also been uncommonly attributed to tocilizumab therapy and this appears to resolve with discontinuation of the agent. The incidence of viral, bacterial, fungal and mycobacterial infection is modestly increased in patients receiving chronic therapy for rheumatologic disease, thus providing the basis for a black box warning associated with the agent⁴². We have not observed acute infusional toxicities secondary to tocilizumab in patients treated on our studies.

In patients with CRS who respond to tocilizumab, fever and hypotension often resolve within a few hours, and pressors and other supportive care measures can be weaned quickly thereafter. In some cases however, symptoms may not completely resolve and continued aggressive support may be necessary for several days (Case #3). If the patient's condition does not improve or stabilize within 24 hours of the tocilizumab dose, administration of a second dose of tocilizumab and/or a second immunosuppressive agent, such as corticosteroids, should be

considered. Whether lack of response is related to ongoing production of IL-6, inadequate dosing of tocilizumab, or other factors is not yet known.

As discussed above, neurologic symptoms associated with CRS sometimes follow a different time course of onset and resolution (Case #3). We have occasionally witnessed patients whose hemodynamic instability resolves rapidly following administration of tocilizumab, but who subsequently develop signs and symptoms of neurotoxicity. Based upon the following evidence, we hypothesize that this relates to IL-6 directed neurotoxicity. First, IL-6 has been reported to mediate substantial neurologic effects and has been implicated in several neurologic conditions spanning Alzheimer's disease, Parkinson's disease, multiple sclerosis, schizophrenia, depression and MERS.³⁵⁻³⁷ Second, elevated CNS IL-6 levels are likely to occur in CRS due to direct transit of IL-6 via production in the periphery, and/or production of IL-6 via trafficking of activated immune cells to the CNS. Indeed, we have observed elevated IL-6 levels in the CSF associated with neurotoxicity. Third, IL-6 levels rise transiently following administration of tocilizumab due to blockade of the receptor⁴⁷, which inhibits receptor-mediated clearance and tocilizumab is not expected to cross the blood:brain barrier. Hence, elevations in IL-6 within the CNS are not likely to be directly ameliorated in the short-term and may even be transiently aggravated by tocilizumab therapy. For these reasons, for patients with Grade 3 or 4 CRS associated with neurologic dysfunction without significant hemodynamic instability or other life-threatening symptomatology, consideration may be given to the use of corticosteroids as a preferred first line immunosuppressive.

Figure 3 depicts a hypothetical patient with grade 3 CRS who develops fever on Day 3, then hypotension on Day 5 that is initially managed with a low-dose vasopressor, then tocilizumab on Day 6 after cardiovascular decompensation. IL-6 and IFN γ peak coincident with

decompensation. IFN γ rapidly returns to baseline after tocilizumab, but owing to its mechanism of action, whereby receptor mediated endocytosis of IL-6 is blocked by tocilizumab, IL-6 levels typically rise transiently immediately following tocilizumab before returning to baseline. Numerous other cytokines are also elevated and follow a similar time course. As depicted, neurologic symptoms can manifest either before or after tocilizumab administration, but not infrequently persist for some time after tocilizumab, presumably due to its inability to cross the blood brain barrier.

Corticosteroids and other agents

Clinical experience also demonstrates that corticosteroids are effective treatment for CRS, and a rapid steroid taper can generally be accomplished within several days without recurrence of the CRS. Despite this, we currently consider corticosteroids as second line therapy for CRS. This is based upon our clinical observation that response to tocilizumab may be more rapid than response to corticosteroids. In addition, corticosteroids appear to have more widespread effects on the immune system and emerging evidence that suggests they may mediate a greater adverse effect on the antitumor activity of adoptively transferred T cells²⁶. We acknowledge however, that the recommendation to use tocilizumab over corticosteroids is based upon limited clinical experience and that in some settings wherein immune toxicity is associated with antitumor therapies, such as graft-versus-host disease and checkpoint inhibitor therapy, corticosteroids do not prevent therapeutic benefit^{1,49}. Thus, some physicians may choose to use corticosteroids as a frontline agent or to utilize both agents in cases of severe or life-threatening CRS. Although dosing and choice of corticosteroid should be tailored to the individual patient, commonly used initial doses include methylprednisolone (2 mg/kg/day), which can generally be

weaned over several days. For patients with severe neurologic symptoms, consideration may be given to using dexamethasone (0.5 mg/kg, max 10mg/dose) due more efficient penetration of the blood brain barrier⁴⁸, although evidence for differential efficacy of methylprednisolone versus dexamethasone in this setting has not been established. It is also important to note that, especially in the case of B-ALL, many patients have chronically received corticosteroids as part of their treatment regimen, and therefore some patients who experience CRS may have a relative corticosteroid deficiency due to chronic suppression of their hypothalamic-pituitary axis wherein stress doses of hydrocortisone may be indicated.

Targeted immunosuppressive agents are also available to inhibit TNF α and IL-1, both of which may contribute to CRS. Hence, anti-TNF α mAbs (infliximab) and soluble TNF α receptor (etanercept) and IL-1R based inhibitors (anakinra) could also provide benefit and have been used successfully (Case #2). These agents have also demonstrated efficacy in the setting of macrophage activation syndrome and other syndromes⁵⁰⁻⁵² that likely overlap with CRS in terms of pathophysiology. However, because supportive care with tocilizumab \pm corticosteroids has been efficacious in our experience, we are not currently routinely using these agents.

Conclusion

Modern antitumor immunotherapies show impressive promise, but effective application of this new class of therapeutics requires that clinicians learn to recognize and manage novel toxicities associated with tumor immunotherapy. The overarching goal of CRS management in patients treated with immunotherapy for cancer is to prevent life-threatening CRS while maintaining the greatest chance for a beneficial antitumor effect. We have outlined here a pro-

active management strategy that incorporates a grading system and treatment algorithm designed to administer early immunosuppression for patients at highest risk, while avoiding unnecessary immunosuppression due to the potential risk of diminishing antitumor efficacy. Future work is needed to better understand the pathophysiology of this syndrome, to more definitely delineate the aspects of immune activation required for antitumor effects and to validate optimal treatment strategies. Given the limited clinical experience with the syndrome, consideration could be given to a national or international registry to monitor outcomes and glean further insight into the spectrum of symptomatology and optimal management of CRS. Further, it is important to emphasize that the severity of CRS is greater in patients with higher disease burdens. Incorporation of immunotherapy into regimens that administer these therapies to patients with lower disease burdens would be expected to substantially reduce the toxicity observed and potentially benefit patients in the absence of any clinical evidence for CRS.

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Authorship Contributions

D.W.L, R.G, D.L.P., C.U.L, N.A., M.J., S.A.G. and C.L.M. wrote and edited the manuscript.

Conflict of Interest Disclosure

M.J. has ownership interest in Juno therapeutics. D.L.P. has a patent in the field of adoptive immunotherapy and sponsored research support from Novartis. S.G. consults for and received research support from Novartis. C.L.M. has a patent in the field of adoptive immunotherapy and sponsored research support from Neomune. C.U.L. is a member of the Scientific Advisory Boards for TeamConnor, Solving Kid's Cancer, and Kids' Cancer Research Foundation. The Center for Cell and Gene Therapy has a collaborative research agreement with Celgene. N.A.: The Center for Cell and Gene Therapy has a collaborative research agreement with Celgene.

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Table 1. Clinical Signs and Symptoms Associated with Cytokine Release Syndrome

<i>Constitutional:</i> fever ± rigors, malaise, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, headache
<i>Skin:</i> rash
<i>Gastrointestinal:</i> nausea, vomiting, diarrhea
<i>Respiratory:</i> tachypnea, hypoxemia
<i>Cardiovascular:</i> tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late)
<i>Coagulation:</i> elevated d-dimer, hypofibrinogenemia ± bleeding
<i>Renal:</i> azotemia
<i>Hepatic:</i> transaminitis, hyperbilirubinemia
<i>Neurologic:</i> headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, seizures

Table 2. Cytokine Release Syndrome Revised Grading System

Grade 1	<i>Symptoms are not life threatening and require symptomatic treatment only</i> e.g. fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	<i>Symptoms require and respond to moderate intervention</i> -oxygen requirement <40% or -hypotension responsive to fluids or low dose ² of one vasopressor or -grade 2 organ toxicity
Grade 3	<i>Symptoms require and respond to aggressive intervention</i> -oxygen requirement \geq 40% or -hypotension requiring high dose ¹ or multiple vasopressors or -grade 3 organ toxicity or grade 4 transaminitis
Grade 4	<i>Life-threatening symptoms</i> -requirement for ventilator support or -grade 4 organ toxicity (excluding transaminitis)
Grade 5	<i>Death</i>

¹high dose vasopressor doses shown in Table 3.

Table 3. High-Dose Vasopressors (all doses are required for ≥ 3 hours)

Norepinephrine monotherapy	≥ 20 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200 mcg/kg/min
Epinephrine monotherapy	≥ 10 mcg/kg/min
If on vasopressin	¹ Vasopressin + NE equivalent of ≥ 10 mcg/kg/min
If on combination vasopressors (not vasopressin)	¹ Norepinephrine equivalent of ≥ 20 mcg/kg/min

¹VASST Trial Vasopressor Equivalent Equation:

$$\text{Norepinephrine equivalent dose} = [\text{norepinephrine (mcg/min)}] + [\text{dopamine (mcg/kg/min)} \div 2] + [\text{epinephrine (mcg/min)}] + [\text{phenylephrine (mcg/min)} \div 10]$$

Figure Legends

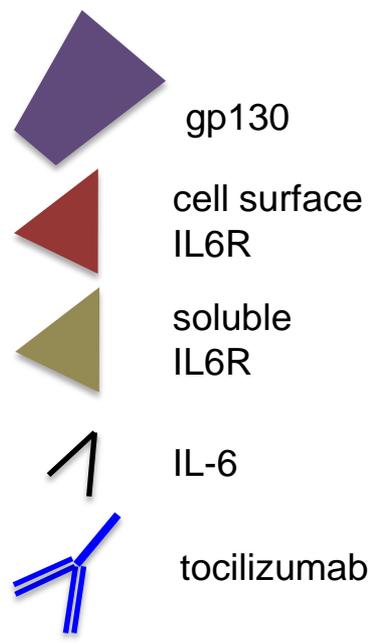
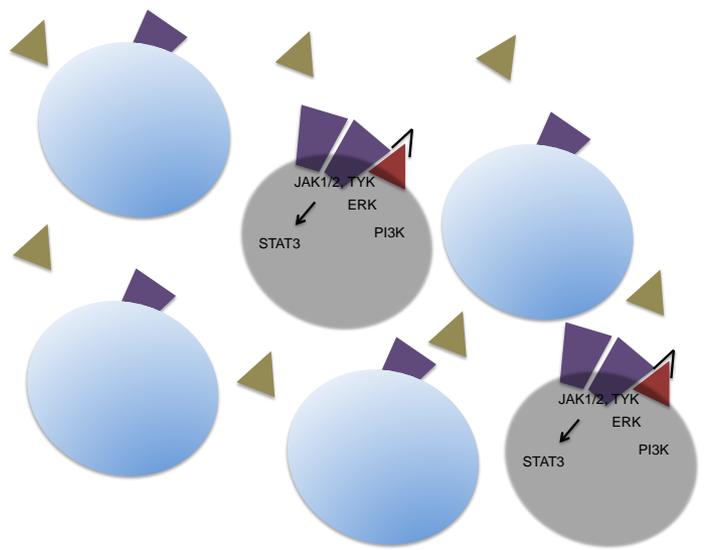
Figure 1. IL-6 Signaling and Inhibition by Tocilizumab. *Panel A* depicts classic IL-6 signaling restricted to IL-6R expressing cells (macrophages, neutrophils, T cells, hepatocytes), which predominates when IL-6 levels are low. IL-6 binds to cell associated IL-6R, leading to homodimerization of gp130 and initiation of downstream pathways. *Panel B* depicts both classic and trans-IL-6 signaling, which occurs when IL-6 levels are elevated, leading to IL-6 signaling on a broad array of cells, since gp130 is ubiquitously expressed. Tocilizumab binding to both cell associated IL6R and soluble IL6R inhibits classic and trans-signaling.

Figure 2. Treatment algorithm for management of CRS based on the Revised CRS Grading System. The algorithm uses the Revised grading system for CRS to direct clinical management for patients with immunotherapy associated CRS. We recommend vigilant supportive care including empiric treatment of concurrent bacterial infections and maintenance of adequate hydration and blood pressure for every grade. Immunosuppression should be employed in all patients with Grade 3 or 4 CRS and instituted earlier in patients with extensive co-morbidities or older age.

Figure 3. Cytokine changes associated with clinical findings in a hypothetical patient with Grade 3 CRS. A dramatic rise in IL-6 and IFN γ levels is associated with the onset of fever at Day 3 after CAR T cell infusion. Despite vigilant supportive care, the patient becomes hypotensive requiring the use of one vasopressor on Day 5. After a brief period of

cardiovascular stability, a second vasopressor is required to maintain adequate perfusion on Day 6, at which time tocilizumab is administered. IL-6 levels continue to rise transiently after tocilizumab, since it continues to be produced by and tocilizumab blocks IL-6 receptor-mediated endocytosis. Vasopressor support is gradually weaned over the next 48 hours although neurologic changes may persist or initially manifest after tocilizumab, but eventually resolve. Several other inflammatory cytokines, including TNF α , IL-2, GM-CSF and others noted in the text are also likely to be elevated during the peak of the syndrome.

A. Low IL-6 Levels: Classic IL-6 Signaling



B. High IL-6 Levels: Classic- and Trans-Signaling Inhibited by Tocilizumab

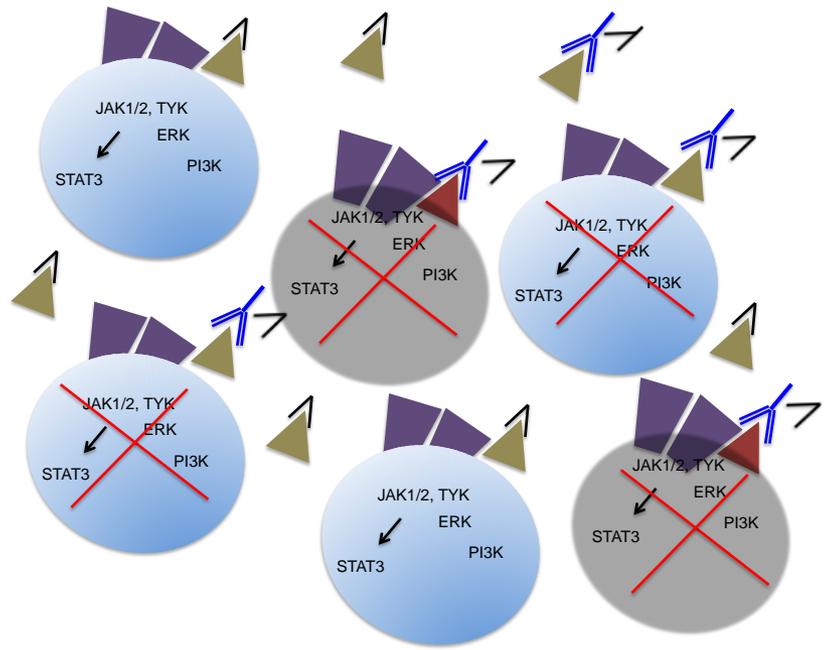


Figure 1

GRADING ASSESSMENT

TREATMENT

Grade 1 CRS

Fever, constitutional symptoms

Grade 2 CRS

Hypotension: responds to fluids or one low dose pressor
Hypoxia: responds to $<40\%$ O_2
Organ toxicity: grade 2

Grade 3 CRS

Hypotension: requires multiple pressors or high dose pressors
Hypoxia: requires $\geq 40\%$ O_2
Organ toxicity: grade 3, grade 4 transaminitis

Grade 4 CRS

Mechanical ventilation
Organ toxicity: grade 4, excluding transaminitis

*Extensive
co-morbidities
or older age?*

No

Yes

- Vigilant supportive care
 - Assess for infection(Treat fever and neutropenia if present, monitor fluid balance, antipyretics, analgesics as needed)

- Vigilant supportive care (Monitor cardiac and other organ function closely)

- Vigilant supportive care
 - Tocilizumab \pm corticosteroids

Figure 2

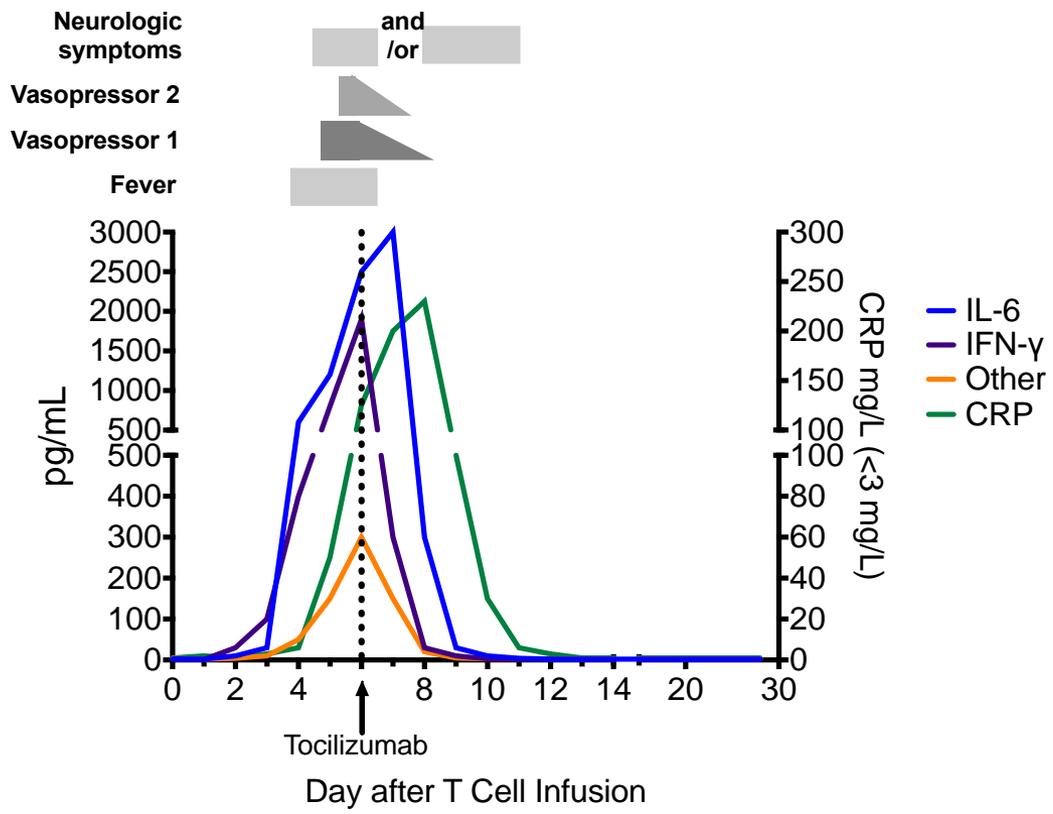


Figure 3