GM-CSF: The Initiator of CAR-T-Induced CRS and Neurotoxicity

Keywords
Cytokine Release Syndrome (CRS)
Neurotoxicity
GM-CSF
Inflammatory Cascade
Self-Reinforcing Feedback Loop
Chimeric antigen receptor T cell (CAR-T) therapy utilizes a patient’s immune system to recognize and attack a specific tumor antigen. The results of CAR-T clinical trials have shown high remission rates, including remission in radiation- and chemo-unresponsive patients. However, the ‘miracle cure’ comes at a high risk. CAR-T-induced neurotoxicity (NT) and cytokine release syndrome (CRS) require intensive hospital monitoring and have resulted in deaths. To get the most out of the exciting innovation of CAR-T, it is imperative to reduce these severe and life-threatening side effects. This review, synthesizing key primary research investigating NT and CRS, illustrates how GM-CSF appears to be central to the inflammatory cascade. Neutralization of GM-CSF has the potential to not only abolish NT and CRS, but also improve the efficacy of CAR-T therapies.

**Significance**

- Clinical trials have illustrated the power and significance of CAR-T therapy in treating several malignant cancers. The FDA has approved two CAR-T products.
- CAR-T-induced neurotoxicity and CRS are life threatening conditions. An ability to neutralize the inflammatory cascade is critical for the future success of patient treatment. GM-CSF is the key initiator of the self-reinforcing inflammatory pathway of neurotoxicity and CRS.
- Lenzilumab, an anti-GM-CSF monoclonal antibody, has the potential to improve the clinical management, patient outcomes, and therapeutic index of CAR-T. This could enable administration of higher CAR-T doses and reduce the current hospitalization bottleneck, allowing more patients to be treated.

**Glossary**

**CAR-T Therapy for Cancer**: A process in which T cells extracted from a patient are engineered to recognize epitopes on cancerous cells and eradicate them with high specificity when reintroduced into the patient.

**Cytokine**: A vast family of small proteins crucial in intercellular signaling and inflammatory responses from the innate and adaptive immune system. They are secreted from both B and T cells as well as macrophages and mast cells. Cytokines can influence the production and activity of other cytokines.

**Cytokine Release Syndrome (CRS)**: A serious condition and life-threatening adverse effect because of abnormal cytokine regulation and thus, high inflammation. Symptoms include fever, increased heartbeat and breathing, nausea, vomiting and seizures.

**Neurotoxicity**: Damage to the central or peripheral nervous system. Neurotoxicity can be enhanced by endothelial activation and vascular permeability.

**GM-CSF**: Granulocyte-macrophage colony-stimulating factor, a cytokine that stimulates monocyte and granulocyte production and acts as a recruiter in the inflammatory cascade.

**Antibody**: Secreted by B cells, immunoglobulins bind a unique epitope highly specifically to both neutralize an antigen and mark it for effectors of the immune system.
Introduction

Immunotherapies are a new generation of cancer treatments, poised to radically change clinical oncology with their specificity and high response rates. CAR-T therapy, a new form of immunotherapy, has been approved twice by the FDA to date with over 300 clinical trials currently in process. High remission rates are being observed in cancers unresponsive to radiation and chemotherapy. However, CAR-T cell therapy is currently limited by the risk of life-threatening neurologic toxicity and CRS. Despite active management, all CAR-T responders experience some degree of CRS. Up to 50% of patients treated with CD19 CAR-T have at least Grade 3 CRS or neurotoxicity. Novartis’ CAR-T therapy, Kymriah had an 83% remission rate in acute lymphoblastic leukemia (ALL) after three months. However, over the course of the Novartis trial, nearly half of all patients suffered from moderate to severe cases of CRS. Kite Pharmaceuticals’ CAR-T trial resulted in two CRS- and neurotoxicity-related deaths. Juno Therapeutics terminated a trial after neurotoxicity resulted in cerebral edema and death in five patients. CRS and neurotoxicity are limiting the number of patients treated by CAR-T therapy and the dosage administered.

Reducing or eliminating CRS and neurotoxicity in these therapies would provide great value. Determining what is driving or exacerbating the signature CAR-T inflammatory response is a crucial first step. Although a plethora of cytokines, signaling molecules, and cell types are involved in this pathway, one cytokine appears to be at the heart of CRS and neurotoxicity, GM-CSF. Normally undetectable in human serum, it is central to the cyclical positive feedback loop that drives inflammation to the extremes of cytokine storms and endothelial activation. Neurotoxicity and cytokine storms are not the result of a simultaneous release of cytokines, but rather a signaling cascade of inflammation initiated by GM-CSF that results in the trafficking and recruitment of myeloid cells to the tumor site. These myeloid cells produce the cytokines observed in CRS and neurotoxicity, perpetuating the inflammatory cascade.

The Central Role of GM-CSF

CRS involves the increase of several cytokines and chemokines including IFN-γ, IL-6, IL-8, CCL2 (MCP1), CCL3 (MIP1α), and GM-CSF [1,2]. IL-6, one of the key inflammatory cytokines, is not produced by CAR-T [3]. Instead, it is produced by myeloid cells which are recruited to the tumor site. GM-CSF mediates this recruitment, which
induces chemokine production that activates myeloid cells and causes them to traffic to the tumor site. Specifically, the Rosenberg lab demonstrated that GM-CSF and IL-15 peak in the early stages of CRS, suggesting their roles as initiators (see Figure 1) [4]. Early GM-CSF increases correlate with more severe CRS cases [5].

Elevated GM-CSF levels serve as both a predictive biomarker for CRS and an indicator of its severity. Results from Locke et al. support this postulation, illustrating that IL-2, GM-CSF and ferritin are the key immune signatures of neurotoxicity [6]. Furthermore, excess GM-CSF production is sufficient in vivo to induce neurologic effects and brain inflammation [7]. The evidence produced thus far suggests GM-CSF plays a central role in CRS and neurotoxicity.

**Executive Overview**

- IL-15 and GM-CSF peak early in the CRS process.
- IL-6 is not produced by CAR-T.
- GM-CSF knockout CAR-T recruit fewer NK cells, CD8 cells, myeloid cells, and neutrophils to the tumor site in comparison to CAR-T.
- Murine IL-6 knockouts still get CRS; IL-6 is not the initiator of this inflammatory cascade.
- GM-CSF knockout CAR-T treated mice have normal levels of INF-γ, IL-6, IL-10, CCL2 (MCP1), CCL3/4 (MIG-1).

More than a critical component of the inflammation cascade, GM-CSF is the key initiator, responsible for both CRS and neurotoxicity. In vivo studies using murine models indicate that genetic silencing of GM-CSF prevents cytokine storm – while still maintaining CAR-T efficacy [8]. GM-CSF knockouts have normal levels of INF-γ, IL-6, IL-10, CCL2 (MCP1),
CCL3/4 (MIG-1) in vivo and do not develop CRS [9]. Summarized in Figure 2, IL-6 knockouts still develop CRS, demonstrating that IL-6 is not the initiator of this inflammatory cascade. The clinical significance in this observation is that it reveals a potential mechanism for a prophylactic approach to neurotoxicity and CRS. Currently the only treatment for CRS is the anti-IL6 tocilizumab – which is not effective for neurotoxicity. GM-CSF knockout CAR-T models reduce CRS by recruiting fewer NK cells, CD8 cells, myeloid cells, and neutrophils to the tumor site in comparison to normal CAR-T [11]. Ablation of GM-CSF is both necessary and potentially sufficient to prevent CRS and neurotoxicity.

**CRS and Neurotoxicity: How GM-CSF Initiates the Cascade**

The mechanism by which GM-CSF initiates the inflammatory cascade is also becoming clear. A key component of the cascade is its ability to self-perpetuate, a phenomenon for which the CCR2+ myeloid cells are largely responsible. GM-CSF is the driver causing CCR2+ myeloid cells to traffic to the site of inflammation and initiating a self-perpetuating inflammatory cascade within tissues, including the central nervous system (CNS) [12]. The CNS is particularly vulnerable to monocyte-derived phagocytes and GM-CSF drives the activation, trafficking, and recruitment of these myeloid cells [7]. CCR2+ myeloid cells produce CCL2 (MCP1) and possess a receptor for CCL2, a critical
chemokine in signaling the cascade. Thus, CCL2 positively mediates its own production in a self-reinforcing manner. After recruitment to the tumor site, myeloid cells produce IL-1 and IL-6 which induce further GM-CSF production by CAR-T [3]. GM-CSF stimulates cytokine production from myeloid cells, resulting in a positive and cyclical signaling pathway of inflammation. Preventing myeloid cell recruitment by GM-CSF is crucial in preventing CRS and neurotoxicity. It has been demonstrated that tumor sites of GM-CSF knockout CARs produce less CCL2 (MCP1), CCL5, and CCL3 (MIP1) [9]. These are key chemokines for both trafficking and recruitment of myeloid cells. A pictorial summary of GM-CSF's role in recruiting myeloid cells and initiating the inflammatory pathway of CRS and neurotoxicity can be seen in Figure 3.

Figure 3. The Central Role of GM-CSF in CRS and Neurotoxicity. Perforin allows granzymes to penetrate the tumor cell membrane. CAR-T produced GM-CSF recruits CCR2+ myeloid cells to the tumor site, which produce CCL2 (MCP1). CCL2 positively reinforces its own production by CCR2+ myeloid cell recruitment. IL-1 and IL-6 from myeloid cells form another positive feedback loop with CAR-T by inducing production of GM-CSF. Phosphatidylserine is exposed as a result of perforin and granzyme cell membrane destruction. Phosphatidylserine stimulates myeloid cell production of CCL2, IL-1, IL-6, and other inflammatory effectors. The final outcome of this self-reinforcing feedback loop results in endothelial activation, vascular permeability, and ultimately, CRS and neurotoxicity.
In summary, infused CAR-T produce perforin, which allows granzymes to penetrate the tumor cell membrane. CAR-T also produce several chemokines and cytokines, including GM-CSF. GM-CSF recruits CCR2+ myeloid cells to the tumor site, which begin to produce CCL2 (MCP1). After GM-CSF activation, CCR2+ myeloid cells traffic to the site of CAR-T activation and secrete a number of inflammatory cytokines, including IL-1, IL-6, IL-8, and GM-CSF; they also recruit other myeloid cells through CCL2 production, thus amplifying a self-perpetuating inflammatory cascade. CCL2 positively reinforces its own production. IL-1 and IL-6 form another positive feedback loop with CAR-T by inducing production of GM-CSF. Meanwhile, tumor cells are being killed via destruction of the cell membrane by perforin and granzymes. As a result, the interior layer of the phospholipid bilayer including phosphatidyl-serine is exposed. This process also stimulates myeloid cell production of CCL2, IL-1, IL-6, and other inflammatory effectors. The final outcome of this self-reinforcing feedback loop results in endothelial activation, vascular permeability, and ultimately, CRS and neurotoxicity. Furthermore, severity of neurotoxicity correlates with higher peak concentrations of IL-6, CCL2 (MCP1), and GM-CSF [5].

Monoclonal Antibody Targeting of GM-CSF and Concluding Remarks

GM-CSF is a key trigger for cytokine storm following CAR-T therapy. Cytokine storm is a self-perpetuating inflammatory cascade resulting in life-threatening side effects. An anti-GM-CSF antibody holds great promise for reducing neurotoxicity, for which there is no treatment, as well as CRS, and potentially increasing safety and efficacy of CAR-T. Lenzilumab, a novel, first-in-class monoclonal antibody, binds to and neutralizes circulating GM-CSF. Shown to be safe and tolerable in several previous clinical trials, lenzilumab is also a potent inhibitor of GM-CSF in vivo. The initial trigger of cytokine and chemokine production that recruits myeloid cells potentially can be blocked by antagonizing GM-CSF. Inhibiting myeloid cell recruitment and trafficking could significantly reduce CCL2, IL-1, and IL-6, which could inhibit the self-perpetuating inflammatory cascade (Figure 2).

CAR-T cell therapy is currently limited by the risk of life-threatening CRS and neurologic toxicity. These side effects limit both the number of patients that can be treated with CAR-T and the dosage that can be safely administered. They also require intensive hospital monitoring of CAR-T patients during therapy. Lenzilumab could aid in the expansion of CAR-T by allowing for a higher CAR-T dose, potentially improving efficacy and reducing patient hospitalization. Hospitals now must ensure that a place in the ICU is available for each CAR-T patient prior to treatment, limiting the potential for true outpatient administration and adding significant costs and patient management hurdles to the therapy. Current treatment for CRS mostly revolves around tocilizumab, an IL-6 receptor antagonist, which treats some but not all cases of CRS, but is not effective as a prophylaxis or treatment of neurotoxicity. An anti-GM-CSF approach with lenzilumab could
prevent neurotoxicity and CRS, a potential key differentiator in clinical practice. Lenzilumab is poised to not only improve the clinical management and patient outcomes of CAR-T cell therapy, but also significantly reduce hospitalization costs, while enabling the treatment of more patients.

References


Please note: this is a company analysis of existing primary research illustrating the scientific rationale for developing lenzilumab for the potential prophylaxis of CAR-T-induced side effects. This rationale is still unproven in clinical studies.