

CAR-T cell therapy – toxicity and its management

Article history:

Received: 09.01.2020

Accepted: 14.01.2020

Abstract

Chimeric antigen receptor T-cell (CAR-T) therapy is an effective new treatment for hematologic malignancies. Two anti-CD19 CAR-T products, namely axicabtagene ciloleucel and tisagenlecleucel, have been approved for the management of relapsed/refractory large B-cell lymphoma after two lines of systemic therapy. Additionally, tisagenlecleucel is indicated for refractory acute lymphoblastic leukemia in pediatric patients and young adults up to 25 years of age. CAR-T cells are undoubtedly a major breakthrough therapy in hematocology resulting in up to 90% response rate with durable remissions in population with refractory high-risk disease. However, there are serious side effects resulting from CAR-T therapy, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Manifestations of CRS mostly include fever, hypotension, hypoxia, and end organ dysfunction. Neurologic toxicities are diverse and include encephalopathy, cognitive defects, dysphasia, seizures, and cerebral edema. Since the symptoms are potentially severe, practitioners need to familiarize themselves with the unique toxicities associated with these therapies. In this article, we present a practical guideline for diagnosis, grading and management of CRS and CAR-T neurotoxicity. In addition, infectious complications and late toxicities including prolonged cytopenias and hypogammaglobulinemia are discussed.

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Keywords:

chimeric antigen receptor T cell, diffuse large B-cell lymphoma, cytokine release syndrome, neurologic toxicities, infection

Chimeric antigen receptor T (CAR-T) cells can produce durable remission in hematological malignancies, not responding to standard therapy. In the setting of aggressive lymphoma, two CAR-T products are currently commercially available, namely, axicabtagene ciloleucel (Yescarta, Gilead/Kite), and tisagenlecleucel (Kymriah, Novartis). The treatment with CAR-T cell is associated with unique array of toxicities, which may limit its use. On the basis of registration studies, the most common complications include cytokine release syndrome (CRS), neurotoxicity now referred as immune effector cell-associated neurotoxicity syndrome (ICANS), infections, tumor lysis syndrome, allergic reactions, prolonged neutropenia, and hypogammaglobulinemia. Herein, we present current management recommendations concerning CRS, ICANS, and infections (including also prolonged neutropenia or hypogammaglobulinemia).

Cytokine release syndrome

CRS is the most common complication observed after CAR-T cells infusion. In the registration study ZUMA-1, CRS occurred in 93% of patients, in JULIET study in 67% of patients; however, grade 3–4 was seen in about 11%. The time of appearance of CRS is 2 (1–12) days with an average duration of 3 days for Yescarta and 3 (1–10) days and 7 days duration for Kymriah [1, 2]. The diagnosis of CRS is primarily based on clinical assessment. There are four main symptoms of CRS, namely, fever, hypotension, hypoxia, and end organ toxicity (Tab. I) [3, 4]. The intensity of CRS can be determined by grading system – currently

recommended ASTCT (American Society for Transplantation and Cellular Therapy) grading system replaced previously used Lee grading model, UPenn scale, MSKCC criteria, or CARTOX [4]. Clinical symptoms and signs of CRS with grading model are presented in table I [5].

CRS is a generalized immune response with positive feedback between cytokines and immune system cells and is potentially fatal complication. CRS was first described by John Ferrara over 25 years ago in patients after allogeneic stem cell transplantation with graft versus host disease. It can also be observed in a course of Ebola fever, influenza, smallpox, and acute respiratory distress syndrome. After CAR-T therapy, the cytokines implicated in CRS are directly produced by infused CAR-T cells (activated when their CARs engage the designated antigen), macrophages/monocytes, and dendritic cell. Serum concentrations of wide variety of cytokines include interleukin-1 (IL-1), interleukin-6 (IL-6), soluble IL-6 receptor, interleukin-2 (IL-2), soluble receptor IL-2, tumor necrosis factor alfa (TNF- α), interleukin-10 (IL-10), interleukin-8 (IL-8), interferon gamma (IFN- γ), monocytes chemoattractant protein 1 (MCP-1), granzyme B, and soluble gp130 increase, which can be related to CRS severity. Predictive factors for CRS include three main groups [6, 7]:

- Patient-related factors: infection or inflammatory state, HCT-Cl index
- Tumor-related factors: disease type, tumor burden
- CAR-T related factors: CAR-T cell design (costimulatory domain use) and number in the product

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Table I. ASTCT CRS consensus grading [5]

CRS Grade	Defining Features of Grade
Grade 1	Temperature $\geq 38^{\circ}\text{C}$ Hypotension: none Hypoxia: none
Grade 2	Temperature $\geq 38^{\circ}\text{C}$ Hypotension: not requiring vasopressors Hypoxia: requiring low-flow nasal cannula (oxygen ≤ 6 L/min) or blow-by
Grade 3	Temperature $\geq 38^{\circ}\text{C}$ Hypotension: requiring a vasopressor with or without vasopressin and/or Hypoxia: requiring high-flow nasal cannula (oxygen > 6 L/min), facemask, nonrebreather mask or Venturi mask
Grade 4	Temperature $\geq 38^{\circ}\text{C}$ Hypotension: requiring multiple vasopressors (excluding vasopressin) Hypoxia: requiring positive pressure (e.g. CPAP, BiPAP, intubation and mechanical ventilation)

Organ toxicities associated with CRS may be graded according to CRCAE v5.0 but do not influence CRS grading. Fever is defined as $\geq 38^{\circ}\text{C}$ not attributable to any other cause.

New predictive markers are being sought to predict and monitor CRS more precisely. Current CRS monitoring markers in clinical practice include ferritin, C-reactive protein (CRP), IL-6, IFN- γ . Ferritin and CRP are the most relevant due to the availability and speed of determination.

Recommendation

- CAR-T cells should not be administered in case of symptoms of infection. In the case of infection, administration of the product should be postponed.
- Patient should be monitored daily for the first 10 days following CAR-T infusion for signs and symptoms of potential CRS.
- After the first 10 days, the patient should be monitored at the physician's discretions. Patient should be instructed to remain within proximity of the hospital for at least 4 weeks following infusion.
- To confirm the diagnosis of CRS, other causes of systemic inflammatory response must be excluded, including infections. In the case of neutropenic fever or documented infection treatment with broad-spectrum antibiotics, fluids, and maintenance treatment must be used in accordance with the existing medical indications.
- Therapy of CRS is related to its grade and includes symptomatic treatment, tocilizumab and corticosteroids. Details are described in table I. From grade 3, treatment on intensive care unit (ICU) is recommended.
 - Grade 1 – only symptomatic treatment: pain killers, anti-emetics, antipyretics, sedatives, and so on.
 - Grade 2 – symptomatic treatment: fluid therapy, oxygen therapy + tocilizumab at a dose of 8 mg/kg intravenously for 1 hour (do not exceed the dose of 800 mg). If there is no improvement, low RR or oxygen demand increases, repeat the dose every 8 h, up to a maximum of 4 doses. When there is no improvement after 24 h, treatment as in Grade 3 is recommended.
 - Grade 3 – symptomatic treatment: fluid therapy, oxygen therapy + tocilizumab every 8 h. Do not give more than 3 doses in 24 h; the maximum total number of doses is 4, if no clinical improvement in the signs and symptoms of CRS, give methylprednisolone, intravenously at a dose of 1 mg/kg twice

daily or an equivalent dose of dexamethasone (e.g., 10 mg intravenously every 6 h). Continue corticosteroid treatment until the event is classified as a grade 1 or less event, then gradually reduce the dose over 3 days. If there is no improvement, apply treatment as per grade 4. Grade 3 of CRS require treatment on ICU.

- Grade 4 – treatment as in grade 3 + methylprednisolone intravenously at a daily dose of 1000 mg for 3 days, when there is improvement continue treatment as in grade 3, in the absence of improvement or deterioration consider other immunosuppressive drugs.
- The possibility of haemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS) or infections should be considered in patients with severe or nonresponsive CRS treatment.

Tocilizumab is a recombinant humanized monoclonal antibody directed against the IL-6 receptor, registered for the treatment of patients with systemic connective tissue diseases. The drug blocks the signal transmission through the IL-6 receptor, thus inhibiting the inflammatory reaction. Tocilizumab induces the rapid reversal of CRS and has become the standard of care for this complication. Corticosteroids suppress the inflammatory reaction in patients not responding to IL-6 receptor blockade. Preliminary results suggest that the use of IL-6 receptor blockade or steroids is not associated with higher rate of lymphoma relapse compared with patients without CRS after CAR-T. New drugs blocking the signal pathways in CRS are being tested. These drugs include siltuximab – monoclonal antibody anti-IL-6, anakinra – human interleukin-1 receptor antagonist, tyrosine kinase inhibitor – dasatinib, adenosine A3 receptor agonists, JAK-STAT inhibitors (namodenosone, piclidenosone), lenzilumab – monoclonal antibody anti-GM-CSF.

Immune effector cell-associated neurotoxicity syndrome

Immune effector cell-associated neurotoxicity syndrome (ICANS) is the second most common acute toxicity observed with CAR-T cell therapy. The rate of ICANS of any grade associated with CD19-targeted CAR-T cell therapy in patients with lymphoma ranges from 23% to 67%, with grade 3–4 seen in 12–30% [3, 4, 7]. It may

manifest with a wide variety of symptoms, with the most typical encephalopathy with word-finding difficulty, aphasia, and confusion. All neurologic and psychiatric adverse reactions reported in clinical trials with currently approved CAR-T products are listed in table II. Symptoms of ICANS usually occur few days later than symptoms of CRS, but may also occur simultaneously. Very rarely, neurologic toxicities occur in patients who do not have typical signs of CRS. Symptoms of ICANS have been reported to occur 1 day after CAR-T cell infusion, to the third or fourth week after infusion.

The pathophysiology of neurotoxicity is not well understood, as it is for CRS. Similar to CRS, severity of ICANS is associated with patient-specific and tumor factors, including underlying diagnosis, disease burden, pre-existing neurologic comorbidities, treatment history, and patient age. High tumor burden and inflammatory state are associated with more severe toxicity. In addition treatment-related factors, like intensity of lymphodepleting therapy, high CAR-T cell dose, and CAR-T design may also be associated with its severity. Higher neurologic toxicity grade has been observed to be associated with higher grade CRS, suggesting an independent mechanism for each process, but the similar causes. Severe neurologic toxicity is associated with peak blood CRP, early peak of IL-6, and higher blood levels of multiple serum cytokines. Elevated concentration of proteins and cytokines in cerebrospinal fluid (CSF) may reflect enhanced blood/brain barrier permeability which allow CAR-T cells to penetrate central nervous system (CNS). Given all the above, it could be hypothesized that ICANS is caused by the same factors as CRS, but delayed clinical neurological presentation is due to initial

integrity of the blood/nerve or blood/brain barrier respectively which becomes subsequently disrupted [8]. Like CRS, ICANS is completely reversible in most of patients and tends to have a self-limited course. The recommended grading ASTCT model for neurotoxicity is presented in tables III and IV.

Recommendation

- Patients with a CNS disorder are likely to be more vulnerable to develop neurologic events after CAR-T and require special attention.
- The experience of use of CAR-T cells in patients with primary or secondary CNS lymphoma is limited, and thus the risk/benefit has not been established in this population.
- Patient should be monitored daily for the first 10 days following CAR-T infusion for signs and symptoms of potential neurologic events.
- After the first 10 days, the patient should be monitored at the physician's discretions. Patient should be instructed to remain within proximity of the hospital for at least 4 weeks following infusion.
- In case of neurotoxicity grades 1 and 2 imaging of the brain (MRI or in the absence CT) to rule out cerebral edema and electroencephalogram to exclude electrical seizures should be done. Levetiracetam in a dose of 500 mg twice daily is recommended as seizure prophylaxis, started on the day of infusion or after the onset of ICANS.

Table II. Neurologic and psychiatric adverse reactions reported with approved CAR-T [5]

Tisagenlecleucel (Kymriah)	Axicabtagene ciloleucel (Yescarta)
<p>Encephalopathy: includes cognitive impairment, confusional state, depressed level of consciousness, attention disturbances, mental status changes, somnolence, and automatisms</p> <p>Delirium: includes delirium, agitation, hallucination, visual hallucinations, irritability, and restlessness</p> <p>Headache: includes headache and migraine</p> <p>Anxiety</p> <p>Sleep disorder: includes sleep architecture disturbances, insomnia, and nightmares</p>	<p>Encephalopathy: includes encephalopathy, cognitive impairment, confusional state, depressed level of consciousness, disturbed attention, hypersomnia, leukoencephalopathy, mental status changes, paranoia, somnolence, and stupor</p> <p>Delirium: includes agitation, delirium, delusion, disorientation, hallucination, hyperactivity, irritability, and restlessness</p> <p>Headache</p> <p>Dizziness: includes balance disturbances, presyncope, and syncope</p> <p>Aphasia: includes sensory and/or motor aphasia/dysphasia</p> <p>Motor dysfunction: includes muscle spasms, paresis or paralysis</p> <p>Tremor</p> <p>Ataxia</p> <p>Seizure</p> <p>Dyscalculia</p> <p>Myoclonus</p>

Table III. ASTCT ICANS consensus grading [5]

ICANS Grade	Defining Features of Grade
Grade 1	<ul style="list-style-type: none"> • ICE score 7-9 and/or depressed level of consciousness but awakens spontaneously • No seizures, motor weakness, or raised ICP/cerebral edema
Grade 2	<ul style="list-style-type: none"> • ICE score 3-6 and/or depressed level of consciousness but awakens to voice • No seizures, motor weakness, or raised ICP/cerebral edema
Grade 3	<ul style="list-style-type: none"> • ICE score 0-2 and/or depressed level of consciousness but awakens to tactile stimulus • Any clinical seizure focal or generalized that resolves rapidly, or nonconvulsive seizures on EEG that resolve with intervention • No motor weakness • Focal/local edema on neuroimaging
Grade 4	<ul style="list-style-type: none"> • ICE score 0 and patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse or stupor or coma • Life-threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without return to baseline in between • Deep focal motor weakness such as hemiparesis or paraparesis • Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICE – immune effector cell-associated encephalopathy; ICP – intracranial pressure.

Table IV. Encephalopathy assessment tools for grading of ICANS [5]

ICE – immune effector cell-associated encephalopathy
<ul style="list-style-type: none"> • Orientation: orientation to year, month, city, hospital: 4 points • Naming: ability to name 3 objects (eg, point to clock, pen, button): 3 points • Following commands: ability to follow simple commands (eg, "Show me 2 fingers" or "Close your eyes and stick out your tongue"): 1 point • Writing: ability to write a standard sentence (eg, "Our national bird is the bald eagle"): 1 point • Attention: ability to count backwards from 100 by 10: 1 point

- Patients with grade 2–3, ICANS should be treated with dexamethasone in a dose 10 mg intravenously every 6 hours until Grade 1, then taper over 3 days. Levetiracetam should be given as seizure prophylaxis.
- Grade 4 is an indication to treatment on the intensive care unit with and high dose of methylprednisolone 1000 mg per day, for 3 days. Intubation and mechanical ventilation should be considered. Levetiracetam should be given as seizure prophylaxis. Patients with cerebral edema should be managed with hyperventilation, hyperosmolar therapy (mannitol or hypertonic saline) to decrease intracranial pressure, in rare cases ventriculo-peritoneal shunt may be adequate treatment. Levetiracetam, phenobarbital, valproate acid and/or lacosamide should be added in case of seizures. For short-term control, benzodiazepines may be used; EEG monitoring seems to be useful only in patients with remitting confusion or deeply unconscious as they may develop respectively nonconvulsive epileptic seizures or nonconvulsive epileptic state.
- Tocilizumab is recommended only if CRS simultaneously occurred.

Infectious complications

Infectious complications may occur after CAR-T therapy as early or late events. The incidence of these infections is similar to the incidence after other salvage chemoimmunotherapy. Predisposing factors for its development include poor immune function related to malignancy and prior cytotoxic treatment, cytopenia after lymphodepleting chemotherapy, use of corticosteroids or tocilizumab, stay on ICU, hypogammaglobulinemia [9].

Early infections, up to 30 days, are related mainly to neutropenia and are seen in about 23% of patients, based on clinical trials. Most of infections, 80%, occur within the first 10 days, with median 6 (1–27) days from CAR-T cell infusion. Bacteria are the most common pathogen, identified in 17% of patients, mainly as bacteriemia. Viruses, mostly respiratory virus infections, are responsible for about 9% of infections, and fungal infections are rare events (4%). In about 19% of cases, febrile of unknown origin is seen and can be concurrent with CRS. Most of early infections are mild or moderate. The main risk factor for infection development after CAR-T cell infusion is severity of CRS [10].

Between days 30 and 90 after CAR-T infusion, infectious complication is seen in about 14% and is related to hypogammaglobulinemia, prolonged neutropenia (22%) or persistent disease (48%). Viral infections are the most common (respiratory virus infections), followed by bacterial etiology. Invasive fungal infections are observed in high-risk patients with previous allogeneic stem cell transplantation or prolonged corticosteroids therapy.

After day 90, post CAR-T cell administration infections are rare, mild, and treated usually in the outpatient setting. Hypogammaglobulinemia is the most common late event; however, about 16% of patients experience prolonged neutropenia.

Recommendation

- CAR-T cells should not be administered in case of symptoms of infection. In the case of infection, administration of the product should be postponed.
- Patient should be monitored daily for the first 10 days following CART infusion for signs and symptoms of potential infections.
- After the first 10 days the patient should be monitored at the physician's discretions. Patient should be instructed to remain within proximity of the hospital for at least 4 weeks following infusion.
- Total serum IgG concentration should be checked prior to and in monthly intervals after CAR-T infusion for at least 3 months.
- IgG replacement should be considered in patients with IgG < 400 mg/dl and serious, persistent, or recurrent bacterial infections.
- Prophylaxis includes
 - Antibacterial – fluoroquinolones during neutropenia can be considered according to center policy.
 - Antiviral – acyclovir or valacyclovir for 1 year in seropositive patients.
 - Antifungal – for low risk patients fluconazole or micafungin during neutropenia; for high-risk patients posaconazole or voriconazole (prolonged neutropenia, steroids, prior alloHCT).
 - *Pneumocystis jiroveci* – cotrimoxazole from day 28 for 6 months.
 - Vaccinations – live vaccines are contraindicated 6 weeks prior to CAR-T therapy; no data regarding re-vaccination (suggested transplant-like protocol).
- Fever of unknown origin should be treated with broad-spectrum antibiotics, fluids, and other supportive care
- Documented infections should be treated with antibiotics according to general and/or institutional guidance
- Prolonged neutropenia may be treated with G-CSF; however autologous (or allogeneic if prior allogeneic stem cell transplantation) stem cell infusion can be considered.

Summary

CAR-T cells is definitely breakthrough therapy changing the landscape of treatment of refractory B-cell malignancies. Definitely the indications for CAR-T cells will be quickly expanded, and the usage

of this technology will become more common. Enthusiastic results are associated with unique, potentially fatal toxicities. Clinical and laboratory monitoring for CRS and ICANS after CAR-T is imperative for early diagnosis and treatment to avoid early mortality. Grading of severity of these side effects is an essential part of the treatment algorithms and needs to be unified. Tocilizumab and corticosteroids remain the mainstay of the treatment currently. Cytopenias, hypogammaglobulinemia, and infections are also important to be diagnosed and treated as clinically indicated. The CAR-T technology is developing extremely quickly, and new data from conducted clinical trials will certainly change the algorithms of diagnostic and therapeutic procedures.

Authors' contributions

LG, AŁD, MM, AN, DD – paper design; LG, AŁD, MM, AN, DD – manuscript writing. Revision of manuscript – all authors.

Conflict of interest

LG was a participant of Novartis and Gilead Advisory Board, received lecture fee and participated in the meetings organized by Gilead and Novartis.

DD was a participant of Janssen Advisory Board, received lecture fee and participated in the meetings organized by Janssen, received research grants from Janssen.

Financial support

None.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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