



Management of adults and children undergoing CAR t-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE)

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Management of adults and children undergoing CAR t-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE)

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Abstract

Chimeric antigen receptor T-cells are a novel class of anti-cancer therapy in which autologous or allogeneic T-cells are engineered to express a chimeric antigen receptor targeting a membrane antigen. In Europe, Tisagenlecleucel (Kymriah™) is approved for the treatment of refractory/relapsed Acute Lymphoblastic Leukaemia in children and young adults as well as relapsed/refractory Diffuse Large B-cell Lymphoma; Axicabtagene ciloleucel (Yescarta™) is approved for the treatment of relapsed/refractory high-grade B-cell Lymphoma and Primary Mediastinal B-cell Lymphoma. Both agents are genetically engineered autologous T-cells targeting CD19. These practical recommendations, prepared under the auspices of the European Society of Blood and Marrow Transplantation, relate to patient care and supply chain management under the following headings: patient eligibility, screening laboratory tests and imaging and work-up prior to leukapheresis, how to perform leukapheresis, bridging therapy, lymphodepleting conditioning, product receipt and thawing, infusion of chimeric antigen receptor T-cells, short-term complications including cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome, antibiotic prophylaxis, medium-term complications including cytopenias and B-cell aplasia, nursing and psychological patient support, long-term follow-up, post-authorisation safety surveillance, and regulatory issues. These recommendations are not prescriptive and are intended as guidance in the use of this novel therapeutic class.

Introduction

The first experimental attempts to engineer T-cells to express chimeric antigen receptors (CARs) were performed thirty years ago (1, 2). The ultimate goal was to produce functional, high-affinity, chimeric antigen receptor T-(CAR T) cells in which the T-cell receptor (TCR) is re-directed towards a tumour antigen of choice (3). Following refinements in the signalling properties of a CAR within the context of a T-cell, development progressed rapidly from the laboratory to clinical trials and CAR T-cells targeting CD19 now represent a novel and promising therapy for patients with refractory/relapsed B-cell malignancies including acute lymphoblastic leukaemia (ALL) and diffuse large B-cell lymphoma (DLBCL) (4-7, 3). CAR T-cells are also being assessed as treatment for other haematological diseases such as multiple myeloma and acute myeloid leukaemia as well as for solid tumours (8, 9, 5, 10).

Tisagenlecleucel (KymriahTM, previously CTL019, Novartis, Basel, Switzerland) consists of autologous CAR T-cells genetically modified *ex vivo* using a lentiviral vector encoding an anti-CD19 CAR that includes a domain of the 4-1BB co-stimulatory molecule. It is indicated for the treatment of children and young adults up to the age of 25 years with relapsed/refractory B-ALL and was approved by the FDA on 30th August, 2017. It was subsequently FDA-approved on May 1st, 2018, for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy including DLBCL not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma. The European Medicines Agency (EMA) approved similar indications on August 22nd, 2018.

Axicabtagene ciloleucel, (YescartaTM, previously KTE-C19, Gilead, USA) is an autologous CAR T-cell product which has been genetically modified *ex vivo* using a retroviral vector

encoding an antibody fragment targeting CD19 and an intracellular domain including the CD28 co-stimulatory molecule. It was FDA-approved on October 18th, 2017, for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma (PMBCL), high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. The EMA approved its use in relapsed or refractory DLBCL and PMBCL, after two or more lines of systemic therapy, on August 23rd, 2018.

While CAR T-cells are rationally designed targeted therapies, they nevertheless frequently induce life-threatening toxicities that can be mitigated by planning and proper hospital organisation. Comprehensive training should be provided to all categories of personnel including scientists, nurses and physicians, and close collaboration with a range of other specialists, especially intensive care unit (ICU) staff and the neurology/neuroimaging services, is required (11, 12).

As CAR T-cells represent a novel class of therapy and as both of the currently available products have only been evaluated in phase II studies to date, close post-marketing surveillance is mandatory. The EMA has endorsed the use of the EBMT registry for the collection of 15-year follow-up data on treated patients in order to ensure that evaluation of the efficacy and safety of commercially available CAR T-cells continues on an ongoing basis. The Centre for International Blood and Marrow Transplant Research (CIBMTR) fulfils a similar function in the United States of America. The newly updated EBMT Registry Cellular Therapy form is designed to capture the efficacy and side-effects of modern cellular therapies and to provide the required post-marketing surveillance through Post-Authorisation Safety Surveillance (PASS) and other studies. The main objective for professionals in the field is to

evaluate how these innovative treatments compare with the alternative therapeutic options and current standards-of-care. Phase III studies are underway (13).

The clinical use of CAR T-cells is early in its evolution and it is, as yet, unclear whether CAR T-cell therapy constitutes a definitive treatment or whether disease cure will require further immunologically-based consolidation such as allogeneic stem cell transplantation, especially for ALL. Trials on the use of CAR T-cell therapy in DLBCL report long-term disease control in up to 50% of patients. As some of these patients may be cured, allogeneic transplantation as consolidation may not be necessary (14-16). This question can only be answered with longer follow-up.

Research areas include dual antigen targeting to counter one of the most common resistance mechanisms which is loss of the targeted antigen, the inclusion of safety switches such as suicide genes in order to mitigate side-effects when they occur, ‘off the shelf’ allogeneic CAR T products, the refinement of co-stimulatory domains to enhance persistence and avoid immune escape, and the use of non-viral vectors and semi-automated on-site production to simplify the manufacturing process.

Although this field will inevitably change over the coming years, these first EBMT CAR T guidelines aim to provide practical, clinically relevant recommendations for haematologists and other cancer specialists and their teams involved in the administration of CAR T therapies, especially the commercially available products. These guidelines may also be a useful resource for other stakeholders such as pharmacists or health service administrators involved in the planning and delivery of CAR T therapies, given the complexity of their production and administration and their high cost.

Methodology

The Practice Harmonisation and Guidelines subcommittee of the Chronic Malignancies Working Party (CMWP) of the European Society for Blood and Marrow Transplantation (EBMT) proposed the project in December 2018. The EBMT Board accepted the proposal and worked with experts in the field to produce practical clinical recommendations on the management of adults and children undergoing autologous CAR T-cell therapy. A survey was sent to active CAR T centres to solicit feedback on current approaches to the topics covered in these guidelines (17). Their responses (41 of 50 centres) along with a literature review and assessment of both the licensing study protocols and the summaries of product characteristics (SPCs) of the commercially available CAR T products inform these recommendations. Finally, three teleconferences were held in preparation for a two-day workshop that took place in Lille on 4th-5th April, 2019.

These recommendations are intended to reflect current best practice in this novel and rapidly moving field and to support clinicians and other healthcare professionals in delivering consistent, high-quality care. They principally apply to the CAR T therapies that are currently commercially available for the treatment of haematological malignancies. Given the absence of randomised trial evidence in this field, a decision was made not to grade these recommendations. They therefore represent the consensus view of the authors.

Where patients are receiving CAR T therapies on clinical trials, physicians should follow the relevant trial protocols. The management of disease relapse following CAR T therapy is outside the scope of these recommendations.

Patient eligibility for CAR T-cell therapy

The decision to treat a patient with a CAR T-cell therapy should be made collectively at a Multi-Disciplinary Team (MDT) meeting in a designated CAR T centre. The patient's medical history and physical condition are important factors in determining their suitability for treatment.

Trial eligibility criteria and EBMT recommendations are shown in Table 1.

Screening laboratory tests and imaging

Table 2 summarises a recommended minimum set of tests that should be performed at screening in order to assess organ function and patient eligibility.

Work-up prior to apheresis

The current set of rules that apply to human tissue and cell procurement in the European Union derives from the Tissue and Cell Directives published in 2004 (2004/23/EC) and 2006 (2006/17/EC; 2006/86/EC). The EU Commission recently convened a stakeholder meeting to examine whether revision of the Tissue and Cell Directives was required. Although a number of arguments, including manufacturing of Advanced Therapy Medicinal Products (ATMPs), were brought forward in favour of revising the directives, no formal decision has yet been made.

The current rules are solely based on the donor-recipient relationship, whether autologous or allogeneic, and do not address the intended use of the collected material. As a consequence, the same requirements apply both to the collection of mononuclear cells for stem cell transplantation and when procuring the starting material for ATMP manufacturing, unless the Marketing Authorization Holder (MAH) stipulates specific additional requirements.

Cross-border shipment of the collected cell product requires compliance with national regulations both in the country of origin and in the country of destination. Obtaining authorization to export human autologous derived elements will require knowledge of the patient's viral serology.

Table 3 represents a checklist that should be verified before starting the leukapheresis procedure.

How to perform leukapheresis

Scheduling of leukapheresis must be coordinated with the pharmaceutical company as lack of manufacturing capacity is currently one of the bottlenecks in the availability of CAR T-cell therapies (20). Confirmation of an agreed manufacturing slot is therefore mandatory prior to deciding on a date for apheresis. With technical advances and more patients likely to become candidates for these treatments in the coming years, limitations in collection centre capacity are likely to become a challenge.

Any of the commercially available leukapheresis devices are, in principle, suitable for apheresis. While companies may suggest preferences for devices or systems, local experience, local permits and the regulatory approval status of individual devices and systems should guide technology selection. Technically, unmobilized leukapheresis is most similar to apheresis for off-line extracorporeal photopheresis or for the collection of allogeneic mononuclear cells (MNC) intended for post-transplant immunotherapy (Donor Lymphocyte Infusions, DLI); no specific apheresis protocols have so far been proposed by cell processor manufacturers or by the CAR T manufacturers. Proof of proper validation and maintenance of equipment and established training processes for personnel operating or supervising the use of cell processors are key elements required by the Marketing Authorisation Holders in order to qualify and 'on-board' sites that are authorized to collect cells for CAR T-cell manufacturing. Prior accreditation in compliance with the 7th edition of the FACT-JACIE Standards for Haematopoietic Cellular Therapies or FACT Standards for Immune Effector Cells confirm the presence of a pre-existing Quality Management System (QMS), although additional requirements are often identified, including those from pharmaceutical providers and health service commissioners (21).

Further information on the technical aspects of apheresis is provided in the Supplement.

Only one of the commercial CAR T-cell manufacturers – Novartis - currently requires cryopreservation of the mononuclear cells on site. It is stipulated that the White Blood Count (WBC) should be adjusted to $1.0 (0.5-2.0) \times 10^8/\text{mL}$, that an approved (approved by company and local regulators) cryoprotectant be slowly added and that the cells be frozen in controlled-rate freezers prior to storage in vapour phase liquid nitrogen. To produce Kymriah™, Novartis will accept cells that have been harvested within the previous 18 months and cryopreserved with appropriate quality management oversight. Whether autologous blood mononuclear cells intended for CAR T-cell manufacturing should be prospectively collected and cryopreserved in selected patients at high risk of relapse is already under debate. The other commercial manufacturers will collect fresh apheresis product packed in their own specified shipping containers. Until shipping, these apheresis products are stored refrigerated (2-8°C).

Manufacturers' requirements for quality control are currently very limited and may be exceeded by local requirements. There may also be differences between FDA and EMA requirements (22). Accredited and validated testing methods must be used. In addition to infectious disease markers tested in peripheral blood samples on the day-of-collection, reasonable quality control should include sterility testing as well as some hemocytometry (WBC, Haematocrit, CD3^+ , and viable CD45^+). Sampling of the collected cell product must follow the manufacturer's requirements so as not to compromise downstream processing steps, while also adhering to local manufacturing authorizations.

Depending on the disease burden, it may be possible to arrange for leukapheresis before starting salvage chemotherapy to treat disease relapse. There is evidence that cumulative chemotherapy exposure adversely affects the quality of circulating T-cells. Although apheresis can be performed in patients with absolute lymphocyte counts (ALCs) as low as $0.1 \times 10^9/\text{L}$, the likelihood of reaching the target number of autologous lymphocytes and successfully manufacturing the drug product is higher in individuals with ALCs exceeding $0.5 \times 10^9/\text{L}$. In addition, the choice of salvage therapy (chemotherapy, serotherapy and radiotherapy) may adversely affect subsequent attempts at leukapheresis and washout periods need to be considered.

Table 4 provides recommendations on washout periods following various salvage treatments before starting leukapheresis. In addition, it should be noted that prior use of Blinatumomab is not a contra-indication to anti-CD19 CAR T-cell therapy (23).

Bridging therapy

Bridging therapy refers to the administration of anti-cancer drugs including chemotherapy to maintain disease control during the period between lymphocyte collection and the final administration of the CAR T-cell product (16). This time window may be longer than anticipated for logistical reasons, sometimes but not always related to manufacturing, and will be specifically monitored through EBMT Registry collection of 'real world' data.

The goal of bridging therapy is to prevent clinically significant disease progression leading to impaired organ function or any other complications that might prevent the patient proceeding with lymphodepletion and receiving the CAR T-cells. Treatment of rapidly proliferating disease will also hopefully establish a balanced *in vivo* target-effector ratio to allow for effective CAR T adoptive immunotherapy. In brief, the aim is not so much to achieve disease remission as to establish adequate disease control prior to the CAR T infusion.

The optimal bridging therapy for any individual will depend on disease- and patient-specific factors. However, clinicians should bear in mind that patients receiving chemotherapeutic agents, either alone or in combination, will subsequently receive lymphodepleting therapy and will be at risk of specific CAR T-cell-related complications such as cytokine release syndrome (CRS), encephalopathy and tumour lysis syndrome. Bridging therapy should therefore ideally not induce major complications, such as infections, bleeding or any organ dysfunction that might interfere with the planned lymphodepleting therapy and CAR T-cell infusion. Bridging therapy can be omitted in the presence of stable, low burden disease if the turn-around time for the CAR T-cells is expected to be short. Importantly, certain agents, especially immunotherapeutic drugs with a longer half-life, may interfere with the expansion

or persistence of the infused CAR T-cells and should be avoided. Examples include alemtuzumab, daratumumab, checkpoint inhibitors and brentuximab vedotin.

When choosing bridging therapy for lymphoma patients, factors to be considered include the prior response to chemotherapy and chemo-immunotherapy, the overall tumour burden and the distribution and sites of tumour involvement. Options may include parenteral agents such as rituximab, gemcitabine, oxaliplatin, bendamustine or pixantrone; oral chemotherapy regimens e.g. variants of prednisolone, etoposide, procarbazine, and cyclophosphamide (PEP-C), or oral cyclophosphamide 100 mg once daily; novel targeted therapies such lenalidomide or ibrutinib; high dose corticosteroids e.g. dexamethasone 40 mg for four days or high dose methylprednisolone, repeated as needed; or radiotherapy to symptomatic or large masses (24, 25).

In ALL, the risk of CRS has been found to correlate with the leukaemic blast burden at the time of the CAR T infusion. Bridging chemotherapy is therefore especially important in ALL and the chosen agents are typically drawn from known B-ALL chemotherapy regimens though doses are often reduced to reduce the risk of infectious complications and organ dysfunction (5, 26). Novel and targeted agents, for example, tyrosine kinase inhibitors and monoclonal antibodies, may also be used although it is important to consider whether the agent is capable of inducing a rapid response and whether the therapy might interact with subsequent lymphodepleting and CAR T-cell therapy. Whichever treatment is chosen, bridging therapy should only be given after leukapheresis so that the quality of the CAR T-cell product is not affected. Monitoring of the patient after leukapheresis and during and following bridging chemotherapy can occur either at the treating or at the referring centre provided that there are clear lines of communication between the centres regarding the choice

of any treatments and the management of any complications. Frequent monitoring including laboratory testing and imaging is mandatory in order to prevent or rapidly treat complications that might arise while awaiting the arrival of the CAR T product.

Lymphodepleting conditioning

The use of lymphodepleting (LD) conditioning prior to the CAR T-cell infusion creates a 'favourable' environment for CAR T-cell expansion and survival in vivo, probably by eliminating regulatory T-cells (Tregs) (27). In addition, it can lead to the up-regulation of tumour immunogenicity and improve disease control (28). Furthermore, there are data demonstrating that LD conditioning works to promote homeostatic proliferation of adoptively transferred T-cells via increases in the pro-survival/proliferation cytokines, IL-7 and IL-15, and in conjunction with a lack of competition with wild type T-cells (29-31).

Many drugs have been used for LD conditioning including cyclophosphamide, fludarabine, pentostatin and bendamustine as well as total body irradiation (32). In a clinical trial involving 30 patients with B-ALL at the Fred Hutchinson Cancer Research Centre, fludarabine and cyclophosphamide (Flu-Cy) was associated with superior CAR T-cell persistence and better disease-free survival (DFS) when compared to patients who received single agent cyclophosphamide or cyclophosphamide in combination with etoposide (33, 34). 'Flu-Cy' is the most widely used LD conditioning regimen (35, 36).

LD conditioning is usually administered on a three-to-five-day schedule prior to the infusion of the CAR T-cells. If the centre does not have established policies and infrastructure to allow for safe outpatient-based administration, hospitalisation is recommended during this period to ensure close monitoring and optimal hydration.

Items to consider before starting LD are shown in Table 5a.

Laboratory tests to review before starting LD are shown in Table 5b.

If there is a long delay (in general, more than three weeks) between completing LD conditioning and the subsequent CAR T infusion, and the WBC count is $>1.0 \times 10^9/\text{L}$, then

consideration should be given to re-treating the patient with LD chemotherapy prior to receiving the CAR T-cells.

Product receipt and thawing

The currently licensed CAR T-cell products are delivered frozen and must be maintained at very low temperatures during shipping, receipt and temporary storage until they are thawed immediately prior to use. Hospitals have adopted different approaches to product receipt, taking into account local organizational and regulatory issues. The unit receiving the CAR T-cell products will need to have suitable storage containers and facilities for genetically manipulated material; depending on national legislation, a storage site may need regulatory approval as gene therapy medicinal products are also genetically modified organisms (21). As the manufacturing companies use differently sized cryostorage cassettes, custom-made cryo racks, at least one for each company, must be obtained. A storage site with secured access and an adequate number of trained staff licensed to work with biohazards and liquid nitrogen are required, both at the hospital pharmacy and at the cell processing facility.

The designated receiving laboratory will receive advance notice from the manufacturer and the product will be delivered in a sealed liquid nitrogen dewar (vacuum flask). Upon receipt, the seals of the dewar are inspected for breaches; seals are broken, if applicable; the temperature log is read out; and the product is inspected for bag integrity and identity according to the label; the bag in its cassette is subsequently transferred to a liquid nitrogen storage container until it is brought to the bedside. The company-specific product receipt documentation must be completed; personnel authorized to handle products are provided with specific and detailed training from the relevant manufacturer. When the ward is ready to receive the product, the cassette is transferred to a laboratory dewar and this is transported to the ward.

In some countries, the use of water baths, carefully calibrated to 35-37°C, remains acceptable; use of an automated thawing device is preferable. Representative examples of such devices are the SaharaTM (Sarstedt) or PlasmathermTM (Barkey) devices. While the thawing of CAR T-cells is, in principle, the same as for cryopreserved HPC-A, the much smaller volumes of CAR T products only require very short thawing times. We recommend that thawing times be established locally with similarly-sized mock products, ideally with mononuclear cell suspensions in protein-saline-DMSO freezing buffer and testing of post-thaw viability, but at a minimum, with protein-saline-DMSO buffer without cells and observation of the time until the buffer assumes the slushy consistency of a ready-to-spike cryo product. If thawing in a water bath, the spike ports which protrude out of the water must be carefully massaged to ensure they thaw in sync with the rest of the product. The spike ports of the thawed product are uncapped, disinfected and aseptically spiked with the transfusion set, the air trap is filled completely with the cell suspension (no falling drops, as this shears cells) and air is evacuated from the infusion line. The individual responsible for the thawing and preparation of the infusion varies between countries and health care systems. We propose that the decision as to who is responsible should be primarily based on competence, meaning that those individuals who normally thaw autologous transplants are likely best qualified. On this basis, pharmacy, processing facility and clinical transplant staff are all acceptable candidates and bedside thawing is preferable.

Infusion of CAR-T cells

Before starting to thaw the CAR T-cell product, the patient should be assessed. Some factors to consider are shown in Table 6. For the administration of the cells, a transfusion set is required. In general, a typical transfusion filter set with 50-200 μm pore size is used; this is, in fact, mandatory in some countries. Importantly, fluid infusion sets are not suitable due to the sub-micrometer bacterial filters. Transfusion sets with leukocyte depletion filters are also unacceptable. It should be noted that the manufacturers recommend the use of non-filtered tubing sets and our recommendations, and some local regulatory requirements, deviate from this approach.

Pre-medication to prevent adverse reactions is reasonable with the important exception of corticosteroids which may damage the CAR T product; typically, paracetamol derivatives and antihistamines such chlorpheniramine or diphenhydramine are used. Individual guidelines are provided by the manufacturers.

The product is aseptically connected to the port of a central venous catheter. The line to be used for the CAR T-cell infusion must be clearly designated; as with blood and stem cell products, no concurrent medication may be given during the CAR T-cell infusion. Infusion should begin as rapidly after spiking as possible, but no later than 30 minutes thereafter. The small volumes and cell numbers allow for rapid (less than 30 minutes) drip infusion of the cell suspension. The infusion bag and set should be disposed of as biohazard and genetically modified organisms (GMO) waste in compliance with institutional policies and country-specific regulations. Transfusion of the low-volume CAR T-cell product is typically uneventful.

Short-term complications and management: CAR T infusion to Day+28

The rapid *in vivo* proliferation of CAR T-cells may be associated with potential life-threatening toxicities such as CRS and neurotoxicity that generally occur within 14 and 28 days of the CAR T infusion, respectively (37, 11, 36, 38). In addition, LD conditioning may also contribute to the cytopenias.

Hospitalisation

Some centres have established policies and infrastructure that allow for the safe administration of CAR T-cells on an outpatient, ambulatory care basis. However, for ambulatory care to work, clear protocols, staffing and training need to be in place so that patients are able to access a co-ordinator on a 24/7 basis. Centres must also be able to provide both immediate review and the emergency admission of patients under the care of experienced staff. As such arrangements are not currently available in most European centres, we recommend patient hospitalisation during the early post-infusion period unless high level ambulatory care and rapid re-admission pathways are already well established, as in centres already providing ambulatory HCT. Table 7 summarises our recommendation relating to the first 28 days following the CAR T cell infusion. These are in line with a number of clinical trial protocols and the recommendations of scientific societies (21, 39).

Tumour lysis syndrome

CAR T-cell therapy can result in the rapid destruction of tumour cells and therapy-associated adverse events including tumour lysis syndrome (40-42). Standard hospital protocols should apply. Tumour lysis in certain locations (gut, biliary tree, lungs, genitourinary tract) may lead to perforation and the release of commensal organisms leading to peritonitis (43).

Infections

Active infections should be fully treated and under control prior to the administration of LD conditioning and the infusion of CAR T products, especially given the likely cytokine-driven exacerbation of inflammatory processes. The presence of fever should prompt blood and urine cultures, a chest radiograph, and, depending on symptoms, respiratory viral screening, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) nucleic acid testing (NAT), CT imaging, lumbar puncture, and/or brain MRI. Empiric antimicrobial therapy based on symptoms and institutional protocols should not be delayed based on the presumption of CRS and clinicians should consider the prior duration of neutropenia (43).

To reduce the time from recognition of suspected sepsis to treatment with anti-microbial medications, institutions may consider the use of patient group directives or conditional orders. These orders allow nursing staff to respond rapidly to signs and symptoms of infection, an example being the automatic administration of specific IV antibiotics following the detection of a fever.

Cytokine release syndrome

Cytokine release syndrome (CRS) is a form of systemic inflammatory response following the infusion of CAR T-cells. However, CRS has also been described following the administration

of various monoclonal antibodies including bi-specific antibodies and anti-lymphocyte globulin and as a complication of haploidentical transplantation (44-48). CRS is the most common complication after CAR T-cell therapy. Depending on the type of CAR T, the disease characteristics and the grading system which has been used, the reported incidence has ranged from 30-100% and for CRS grade 3 or 4 from 10-30% (49).

The activation of CAR T-cells is the triggering event of CRS. This leads to the release of effector cytokines such as IFN- γ , TNF- α and IL-2. These molecules are, in turn, capable of activating the monocyte/macrophage system and inducing the production of a broad spectrum of pro-inflammatory cytokines (including IL-1, IL-6, IFN- γ , IL-10 and MCP1) leading to a raised CRP and sometimes hyperferritinaemia. In pre-clinical models (humanized immunodeficient mice), it has been shown that human monocytes are the main source of IL-1 and IL-6 during CRS. The syndrome can be prevented by monocyte depletion or by blocking the IL-6 receptor with tocilizumab. Tocilizumab does not, however, protect mice against late lethal neurotoxicity characterized by meningeal inflammation. In contrast, an anti-IL-1 receptor antagonist (anakinra) appeared to prevent CRS and neurotoxicity in animal models (50, 36, 51).

Severe CRS shares clinical features with macrophage activation syndrome (MAS) including fever, hyperferritinaemia and multi-organ dysfunction. CRS usually occurs between 1 and 14 days after the CAR T-cell infusion and can last from 1 to 10 days (52, 11). Its severity is variable and is evaluated according to a novel grading scale recently proposed by an ASTCT consensus panel (38). Rare but fatal cases with neurological involvement have been reported in the literature (11). Risk factors for CRS include tumour burden, the presence of active

infection at time of the infusion, the dose of infused CAR T-cells, the type of CAR T-cell construct and the choice of lymphodepleting regimen (37, 53-55).

The treatment for severe cases, in addition to symptomatic measures, consists of the administration of tocilizumab (monoclonal antibody against IL-6R) and, sometimes, corticosteroids. It should be noted that tocilizumab should be administered no more than four times during one episode of CRS. Siltuximab (monoclonal antibody against IL-6) can be used as a second line treatment (figure 1).

An algorithm outlining the management of CRS is shown in Figure 1.

Neurological toxicity

The neurological toxicity seen in CAR T recipients has recently been termed immune effector cell-associated neurotoxicity syndrome (ICANS), previously termed CRES (38). This is the second most common adverse event following CAR T infusion and its incidence has been reported at rates varying from 12 to 55%. In a recent study of 100 patients, the median time of onset of the first neurologic symptoms was six days (range 1-34 days) after the CAR T infusion (57). The duration of symptoms is generally between two and nine days though late complications may occur (11, 38, 57). In general, it develops either at the same time or following resolution of CRS. Deterioration in hand writing has been shown to be an early predictor of central neurotoxicity. Therefore, daily-writing tests over the first months following the CAR T-cell infusion can be used as a simple tool to detect incipient ICANS.

The spectrum of symptoms and signs is non-specific, ranging from confusion, headaches, tremors, hallucinations and abnormal movements to seizures, papilloedema and coma. Any neurological symptom occurring after the CAR T-cell infusion must therefore be considered as CAR T-related until proven otherwise. However, the ASTCT consensus panel recommended excluding non-specific symptoms such as headache, tremor, myoclonus, asterixis, and hallucinations as they are usually managed symptomatically and do not generally trigger specific interventions.

Severe cases have been reported, occasionally leading to death, due to multi-focal haemorrhage, cerebral oedema and laminar cortical necrosis. The severity is correlated with the increase in specific biomarkers such as CRP, ferritin and IL-6 (58, 59, 11, 60). Close monitoring of patients using validated nursing tools is necessary to identify early manifestations of neurotoxicity. This requires serial cognitive testing.

Rapid access to neurological expertise is needed. Cross-sectional imaging (CT, MRI), electroencephalography (EEG), and CSF examination may all be required in the management of these complex patients. Anti-epileptic prophylaxis with agents such as levetiracetam is not routinely recommended except in patients with a history of seizures or central nervous system disease.

Pre-existing neurological co-morbidities may be a risk factor for the development of ICANS. Disease-associated factors include ALL, tumour burden, history of meningeal involvement and prior CNS-directed therapies (58, 59, 11, 60). The intensity of ICANS has been correlated with the depth of lymphopenia and the homeostatic expansion of CAR T-cells. Moreover, the severity of ICANS has also been found to be associated with the severity and early onset of CRS as measured by the extent of fever within 36 hours of the infusion, hemodynamic instability, tachypnea and hypoalbuminemia reflecting loss of vascular integrity and capillary leakage.

The CARTOX scoring system was updated by the ASCTC consensus panel and has been replaced by the ICE score shown in table 8 (38). A different assessment tool for screening delirium in children is shown in Table 9 and has been adapted from Traube *et al* (61).

Laboratory monitoring of CRS and neurotoxicity

In addition to routine daily haematology and chemistry laboratory tests, CRP and ferritin levels are of use in the monitoring of patients developing CRS and neurotoxicity. Although testing for IL-6 or other cytokine levels are theoretically interesting, cytokine testing is not routinely performed in most centres at present.

Atypical lymphocytes that can mimic blasts are not uncommon at the peak of CAR T-cell expansion and can be found in the peripheral blood, bone marrow, and even the cerebrospinal fluid of patients treated with these therapies. Flow cytometry can be used to exclude relapse. Repeating microbiological testing and imaging to rule out infection is recommended in febrile patients.

Antibiotic prophylaxis

The combined effect of prior treatments (immunochemotherapy and/or autologous or allogeneic HCT, bridging chemotherapy administered after leukapheresis and LD conditioning) all increase the risk of opportunistic infections in patients receiving CAR T therapy. Approximately one third of patients have prolonged neutropenia (beyond day +30) and up to 20% of patients have neutropenia lasting more than 90 days. B-cell depletion and hypogammaglobulinaemia are additional risk factors for infections (63, 64, 15, 16).

After CRS and ICANS, infections are one of the most common side effects of CAR T-cell therapy. Most infections are seen within the first 30 days and are bacterial, and to a lesser extent, respiratory viral infections. Invasive fungal infections are rare and are mostly observed in ALL patients who have undergone prior allogeneic SCT (65).

CAR T-cell recipients, like patients undergoing allo-HCT, are at increased risk of a range of infections at the different stages of their treatment course and appropriate anti-microbial prophylaxis is required. In general, centres performing allo-HCT will be familiar with the care of such patients and there is, as yet, no evidence that there are infectious issues specific to CAR T-cell therapy. Table 10 summarises recommendations for prophylaxis against the most common infections.

There is no evidence to suggest that CMV, EBV or adenoviruses are significant clinical problems after CAR T-cell therapy. Equally, little is known regarding the risk of Hepatitis B and C reactivation as these patients were specifically excluded from the trials. It is not possible to provide recommendations regarding the use of CAR T-cell therapy in patients with HIV infection as seropositive individuals were also excluded. The pharmaceutical companies

may, however, manufacture a drug product for a hepatitis B, hepatitis C or HIV positive patient if the viral load is below the level of detection following treatment. For patients with a history of hepatitis B infection, prophylaxis with tenofovir is recommended (66).

Medium-term complications and management: Day +28 to Day +100

Potential toxicities during this period include delayed tumour lysis syndrome, delayed HLH/MAS and CRS, B-cell aplasia, hypogammaglobulinaemia, graft-versus-host disease, and infections. Neutropenia, thrombocytopenia and anaemia are common though generally slowly resolve over several months. Growth factor support may be indicated in the early stages.

Table 11 summarises tests to be performed during this period and their recommended frequency.

Delayed MAS and CRS

In the ALL CAR T experience, CRS typically occurred between one and fourteen days after the CAR T-cell infusion, whereas in patient with CLL, CRS usually occurred later, between 14 and 21 days after the infusion (42). Regardless of the timing, delayed MAS and CRS are managed using standard approaches.

B cell aplasia and hypogammaglobulinaemia

B-cell aplasia is an almost universal on-target, off-tumour toxicity and results in hypogammaglobulinaemia. It occurs in all responding patients and can persist for several years. This absence of CD19-positive cells correlated with functional persistence of CTL019 cells below the limits of detection of flow cytometry, whereas CTL019 remained detectable by means of quantitative PCR (42). B-cell aplasia can therefore serve as a marker for monitoring CD19-specific CAR-T cell activity over time (42, 67).

Persistent B-cell lymphopenia is associated with sino-pulmonary infections, notably with encapsulated bacteria; consideration can be given to vaccination although there is no evidence and immunoglobulin levels should be monitored (43). It has therefore been standard practice in paediatric centres to administer empiric immunoglobulin replacement following the administration of CAR-T cells. Children with B-cell aplasia should receive immunoglobulin replacement to maintain IgG levels according to institutional guidelines for IgG substitution (i.e. ≥ 5 g/dL) (42). In some cases, this may be a long-term requirement.

There is no consensus regarding systematic supplementation in adults who have been shown to have long-lived CD19-negative plasma cells that continue to confer humoral immunity in patients who were successfully treated with CAR-T cells targeting CD19. Nevertheless, intravenous immunoglobulin replacement is recommended in patients with hypogammaglobulinemia and recurrent bacterial infections with encapsulated bacteria. Patients may transition to home-administered SC immunoglobulins after six months.

GVHD

Donor-derived CAR T-cells may rarely trigger GVHD if harvested from, and then returned to allo-HCT patients. Current evidence suggests that the risk of inducing GVHD with the use of donor-derived CAR T-cells is rare (68-70). However, vigilance is required as this complication is potentially severe and life-threatening. If suspected, GVHD should be diagnosed and managed using standard protocols, balancing the introduction of systemic immunosuppression against its effect on anti-tumour CAR T-cell function.

Infections

Beyond 30 days, viral infections predominate including respiratory viral infections, CMV viremia and pneumonia. Later infections may reflect prolonged immunoglobulin deficiency (up to 46% at day 90) as well as lymphopenia (71). Severe co-infections with CRS include respiratory virus infections (some nosocomial), CMV, human herpes virus-6 (HHV-6) or EBV viraemia, Clostridium difficile colitis, cholangitis, and viral encephalitis (72-74, 67).

Nursing and psychological patient support

CAR T-cells are generally being administered at a small number of regional specialist centres to which patients are referred from general hospitals. Therefore, patients who are treated with CAR T-cells may experience high levels of anxiety due to their prognosis and their new environment. Many will be socially isolated and at a significant distance from their established support networks. The role of the clinical nurse specialist is vital to the success of the procedure as well as providing essential bedside support. Referral to the local counselling/psychology services should be offered to these patients where appropriate.

Patients who are being treated on an outpatient basis and their caregivers should receive comprehensive education on the symptoms of CRS and neurotoxicity and patients should attend the treating hospital without delay in the event that they begin to feel unwell. On discharge, they should be instructed to remain within one hour's travel of the treating hospital for at least four weeks following the infusion, during which time a caregiver should always be present. If the patient lives further away, then alternative accommodation, such as a local hotel or apartment, will be required. Whether lodging at home or in local apartments, ambulatory care arrangements for rapid re-admission should be well established.

All patients must be informed of the potential risks and the precautions that they need to take as described in the relevant product patient information leaflet. They may also receive further written information, according to local practice, in the form of a patient information booklet or leaflet. This should include information and education on the symptoms of CRS and serious neurologic adverse reactions, the need to immediately report any symptoms to their treating physician and the need to remain in close proximity to the centre where the CAR-T cells were administered for at least four weeks following the infusion.

Patients must be advised to keep their Patient Advice Card with them at all times and to show it to any healthcare professional they encounter, especially if they are admitted to another hospital. Patients are advised not to drive for eight weeks after the infusion and only after resolution of any neurologic symptoms. This is due to the risk of delayed neurological toxicity. It is also preferable to have a responsible adult such as a parent, spouse or other caregiver available during the first three months following the infusion. A reliable, consistent and well-informed caregiver is essential.

Long-term follow-up (LTFU) from Day+100 onwards – ‘Late Effects’

Little is known about the long-term effects of CAR T-cell therapy. Only a small cohort of patients has been followed for more than two years. The main identified complications are prolonged cytopenias and hypogammaglobulinaemia. There are also more theoretical concerns about the risk of secondary malignancies and both neurological and auto-immune disease.

It should be recognised that all patients will have been treated previously with multiple anti-cancer therapies, some having also undergone allo-HCT. Some patients may receive CAR T treatment at overseas centres and may then return to a CAR T or HCT centre. There is a duty of care for all CAR T-administering centres to arrange for appropriate local follow-up. In cases of geographical transition, formal communication, including discharge correspondence and other clinical material such as imaging files, should be provided to new healthcare providers.

Protocols and policies (Standard Operating Procedures (SOPs)) for LTFU will need to be put in place. These should cover shared care and out-reach arrangements and should be based on Service Level Agreements (SLAs) between CAR T centres and referring centres.

Multi-disciplinary teams dealing with CAR T therapies should arrange for long-term follow-up of treated patients in order to capture disease status and the late effects of CAR T and prior treatments. The MDT should include a physician with responsibility for CAR T administration, disease-specific specialists, LTFU nursing staff, data managers and clinical trial staff.

LTFU clinics may be incorporated into local arrangements for generic allo-HCT ‘late effects’ clinics with other allo-HCT patients, though dedicated CAR T late effects clinics can be developed if a critical mass of survivors is reached.

The clinic should systematically monitor for the following outcomes

- Disease status – remission, minimal residual disease (MRD), relapse, management of relapse, death
- Further treatments administered post CAR T – including allo-HCT and other immune effector cell therapy/ATMP
- For stable patients in ongoing remission – Three monthly late effects monitoring for first year, annually thereafter or as clinically appropriate
- Infections
- Immunological status – cell markers, immunoglobulins, including CAR T persistence
- New cancers, including secondary myeloid diseases
- New autoimmunity and autoimmune diseases
- Endocrine, reproductive and bone health (including growth and development in children and young adult patients)
- Neurological status (including recovery from ICANS)
- Psychological status and quality of life
- Cardiovascular, including cardiac echocardiographic assessments and risk factors for cardiovascular disease, such as ‘metabolic syndrome’
- Respiratory
- Gastrointestinal and liver

The role of vaccinations following CAR T-cell therapy remains unclear. Until further evidence is available, no specific recommendation can be made. This is, in particular, a problem with small children who might not yet have completed their basic immunization schedule and who therefore need close follow-up.

In view of long term B-cell depletion, the advisability of vaccination and adherence to the standard recommended national schedules needs to be evaluated for each individual based on the history of infections and laboratory assessments of cellular and humoral immunity (75). If vaccines are given, specific antibody responses should be assessed.

Post-authorisation safety surveillance (PASS)

As both tisagenlecleucel (Kymriah™) and axicabtagenequiloleucel (Yescarta™) are the first agents in a novel class of therapies based on the genetic modification of autologous T-cells using viral vectors, the EMA and the FDA have made marketing approval conditional on 15-year PASS. At an EMA-sponsored stakeholder workshop on how to best capture the long-term side effects of different CAR-T products over the next 15 years, it was felt that the reporting of CAR-T safety and efficacy in one European registry would avoid the creation of data silos and would allow for the risks and benefits of the different agents to be transparently compared on a common platform. Such a registry would also set an excellent example as to how public registries could not only improve patient care but also help to support affordable health care (76). In March 2019, the EBMT received a qualification opinion from the EMA which found the cellular therapy module of the EBMT registry to be fit-for-purpose for the regulatory oversight of pharmaco-epidemiological studies concerning CAR T cell therapy (77).

A modified version of the MED-A cell therapy form will be used for CAR T-cells and other academic- or industry-manufactured cell therapies. The data submission time points are Day 0, Day +100, six months, and annually thereafter. This module has already proven to be effective in capturing basic data sets on academic and commercial CAR T infusions, though EMA has requested additional safeguards during data capture for regulatory purposes. However, the current minimal data set requested by EMA for commercial products does not require detailed product information such as CD4 and CD8 ratios or transduction efficiencies, as companies consider these to be sensitive proprietary information. Agreed access to a more detailed data set regarding products being evaluated in clinical trials might benefit all those working in the CAR T-cell research field.

In the United States, the FDA has implemented product-specific Risk Evaluation and Mitigation Strategy (REMS) programs. In parallel, the National Cancer Institute-funded Moonshot Initiative program called Cellular Immunotherapy Data Resource, awarded to the CIBMTR in October 2018, will allow for the collection of real-world data. In recent years, EBMT has worked with CIBMTR to develop common data collection policies so the prospect of robust global datasets on the efficacy and safety of CAR-T cell therapies is on the horizon.

It is expected that patients receiving CAR T therapies in both Investigator-led and Pharma-sponsored trials might also have their follow-up data collected in the EBMT Registry. In order to address concerns pharmaceutical companies may have about the confidentiality of commercially sensitive clinical data, trial data reported to the EBMT registry can be embargoed until investigating centres decide to make such data accessible to the public. Early data collection might also create a virtuous circle whereby knowledge of increased activity might help those lobbying for an improved infrastructure for CAR T across Europe in terms of funding opportunities, regulatory frameworks, and, ultimately, commercial drug approval. EMA approval for the use of the EBMT Registry also places certain responsibilities on EBMT. As a formal data controller, EBMT will need to guarantee a fair and transparent mode of data sharing in order to improve the assessment of these many different agents and ultimately to improve our knowledge on how best to use CAR T therapies.

JACIE and regulatory issues

FACT-JACIE standards were initially developed for the accreditation of HCT programs (78, 79). The current 7th edition of the standards also covers Immune Effector Cells (IEC) to accommodate the rapidly evolving field of cellular therapy, mainly, though not exclusively, genetically modified cells, such as CAR T-cells. FACT-JACIE standards do not cover the manufacturing of CAR T-cells but do include the supply chain and handover of responsibilities where the product is provided by a third party. Specific clauses in the standards detail the following requirements, among others: the need for the appropriate recognition of IEC infusion-related side effects, a policy for the rapid escalation of care in critically ill patients, the availability of specific drugs for CRS and other complications and a labelling system to guarantee both the identification and traceability of the product from the collection to the manufacturer and back to the clinical unit. In all involved areas, there is the need for evidence of adequate staffing and training, satisfactory levels of competency, validated procedures and efficient communication. Documentation is available at www.jacie.org.

During the introductory phase of CAR-T development, some centres received ‘focused’ site visits for IECs. However, now that the 7th edition of the standards is well established, inspection of IEC standards should be routinely incorporated within standard JACIE site visits, particularly as there is much dependency on the wider accreditation requirements of the HCT programme i.e. clinical, apheresis, pharmacy and processing laboratory service, along with quality management system requirements. In fact, in the current 7th edition, only 2% and 6% of items are specifically related to either IEC or HCT, respectively, and 92% of the items are common to all forms of cellular therapy.

In addition to JACIE, the complexity of clinical management of patients receiving CAR-T therapy has led to competent authorities and other regulatory bodies in some European countries requiring the administration of CAR T and other IEC within the context of an accredited allo-HCT programme, where established facilities, staffing and expertise can support most aspects of the CAR T pathway. Regardless, the logistical impact of IEC administration within a HCT program has to be carefully planned; an implementation plan aimed at meeting all accreditation and other regulatory requirements, whilst engaging all professionals, services and infrastructure, is essential. Before starting, an assessment of the number of eligible patients and likely resource requirements will usually have to be reviewed by the competent authorities and other regulators, as well as by funding bodies. As mandated by EMA, the pharmaceutical manufacturers also have their own requirements and routinely inspect facilities before a CAR T programme is commenced.

The EBMT and JACIE expect that most CAR-T activity in Europe will be delivered by experienced allo-HCT centres and, ultimately, as the accreditation cycles of centres roll through to the 7th edition of the standards, the IEC standards will be covered at routine allo-HCT re-accreditation inspections. For the minority of centres that undertake CAR T-cell therapy outside of an accredited allo-HCT programme, there are a number of options. Given that CAR T-cell therapy is presently used predominantly in B-cell Non-Hodgkin Lymphoma, there is the possibility of achieving the IEC standards as part of the accreditation covering autologous HCT (auto-HCT), given that referral for auto-HCT is common in lymphoma practice. The same considerations could also apply to myeloma specialists working outside of allo-HCT programmes, as IEC accreditation standards could be aligned to auto-HCT activity or referral routes routinely established in every myeloma service.

In the event of CAR T or related therapies becoming more broadly applicable to non-haematological cancers and therefore potentially outside of mainstream transplant practice, there are a number of possible routes. Firstly, there may be referral to an accredited HCT programme, where shared care arrangements can be easily accommodated within the quality management systems and service level agreements (SLA). This is a model that already applies to occasional HCT in solid tumours, such as germ cell tumours, where patients are referred back at a mutually agreed, often early, stage post-transplant for ongoing care by the referring medical or clinical oncologists.

An alternative strategy would be to undertake independent IEC accreditation specifically for CAR-T and other IEC. This would have to be an individual decision based upon the number of CAR T patients in a given centre as to whether the establishment of a functional quality system and other generic measures were justified just for CAR T or other IEC. The EBMT and JACIE are currently evaluating the demand and feasibility of this approach, which has been adopted by FACT.

Currently, the general recommendation from the EBMT and JACIE is that CAR T and other IECs are best delivered within the framework of an accredited HCT programme, whether allogeneic or autologous, with shared care policies and SLAs incorporated into the quality systems of the HCT programme. Importantly, JACIE also provides a robust method to ensure that programmes meet the quality and other requirements for mandatory long-term data submission to the EBMT Registry, as well as potential benchmarking of survival outcomes.

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Conflicts of interest

IYA: honoraria from Novartis, Kite/Gilead, and Celgene. He is the Principal Investigator for several clinical trials involving CAR T cell therapies.

CH has received grants from Sanofi, honoraria from Sanofi, Gilead, Novartis, Bellicum and Terumo BCT, and funding for equipment to his institution from Miltenyi and Fresenius.

PB: Research Grants: Neovii, Riemsier, Medac; Advisory Board: Novartis, Cellgene, Amgen, Medac, Servier (Institut); Medac: Patent and Royalties

GWB: honoraria from Novartis and Gilead.

HB: honoraria from Novartis and Celgene and served on the advisory board of Novartis.

FC declares no conflict of interest

SC declares no conflict of interest

RFD: honoraria from Gilead and Celgene.

HE: honoraria from Celgene, Takeda, Janssen, and BMS.

MH: member of the speaker's bureau of Celgene and Gilead. Michael Hudecek is inventor on patent applications related to CAR-T technologies that have been filed by the Fred Hutchinson Cancer Research center (Seattle, WA) and the University of Würzburg (Würzburg, Germany).

MJK is an investigator for several clinical trials involving CAR T cell therapies and has received honoraria from Novartis, Kite/Gilead and Celgene.

UK: honoraria from Novartis, Astra Zeneca and Miltenyi.

JK is inventor on different patents with $\gamma\delta$ T cell receptor sequences, recognition mechanisms, and isolation strategies of TEG and CAR T, scientific advisor and shareholder of Gadeta and received research funding from Miltenyi Biotech, Novartis, and Gadeta.

SM received honoraria from, Celgene, Miltenyi, Kiadis, Bellicum, Jazz Pharmaceuticals and Novartis.

MM declares lecture honoraria and consultant- Novartis, Amgen and Pfizer

JM declares no conflict of interest

AN declares no conflict of interest

SR: Gilead and Novartis Advisory Board and speaker fees

RS declares no conflict of interest

FSG declares no conflict of interest

JAS declares speaker fees from Janssen, Jazz and Gilead and IDMC membership from Kiadis Pharma. He is the Chair of NHS England Specialised Commissioning Clinical Reference Group for BMT

MS declares no conflict of interest

JS: honoraria from Novartis and Gilead

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NK: honoraria from Novartis and Celgene

Contribution

IYA, PH, HB, AN, RS, FSG, JS and AJS designed the research and wrote the methodology chapter and the first draft; NK, PH and IYA wrote the introduction; PH, RFD and GBW wrote the patients eligibility chapter; PH and ST wrote the blood tests and imaging chapter; CC and HB wrote the chapters on apheresis readiness, apheresis, product receiving, storage, transportation and thawing; FC and HE wrote the bridging therapy chapter; AN and MM wrote the chapter on lymphodepletion conditioning, IYA, PH, JM and SC wrote the short-term complications chapter; JS, JM, PH and IYA wrote the chapter on medium-term complications; MJK wrote the chapter on antiinfective prophylaxis; JK, FSG and JAS wrote the chapters related to long-term follow-up and JACIE and regulatory issues. All authors proofed and approved the final manuscript.

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Characteristics	ELIANA (ALL Kymriah™)	JULIET (DLBCL Kymriah™) ≥18 years	ZUMA-1 (High grade B-cell NHL Yescarta™) ≥18 years	EBMT recommendations	Comment
Age limit (NHL)	N/A	SPC - No data are available on children below 18 years of age	SPC - No data are available on children below 18 years of age	No upper age limit	Decision should be based on physical condition rather than age
Age limit (ALL)	'Age 3 years at the time of screening to age 21 years at the time of initial diagnosis' SPC- up to 25 years of age	N/A	N/A	Follow SPC	Ability to collect sufficient cells by apheresis can be a limiting factor in infants and small children
ECOG Performance Status	Karnofsky (age ≥ 16 years) or Lansky (age < 16 years) performance status ≥ 50 at Screening	ECOG performance status that is either 0 or 1 at screening	ECOG performance status of 0 or 1	>2 not recommended Note, however, that real-world data with Yescarta™ included patients with ECOG>2(18)	Prognosis may be less poor if the decline in performance status is due to active disease
History of malignancy	Prior malignancy, except carcinoma <i>in situ</i> of the skin or cervix treated with curative intent and with no evidence of active disease	Previous or concurrent malignancy except adequately treated BCC or SCC, <i>in situ</i> cancer of the breast or cervix treated and without recurrence for 3 years, primary malignancy resected and in remission for more than five years	History of malignancy other than nonmelanoma skin cancer or carcinoma <i>in situ</i> (e.g. cervix, bladder, breast) or follicular lymphoma unless disease free for at least three years	Absence of history of malignancy other than carcinoma <i>in situ</i> (e.g. cervix, bladder, breast) unless disease-free and off therapy for at least three years	
Prior allo-HCT	Not excluded; however, excluded if grade II-IV acute or extensive chronic GVHD	Excluded	Excluded	Not a contra-indication	Active GvHD is listed as a reason to delay treatment in the Kymriah™ and Yescarta™ SPC
Prior anti-CD19/anti-CD3 BiTE antibodies or any other CD19 therapy	Excluded Not a contra-indication as per SPC	Excluded	Excluded if prior CD19 targeted therapy	Not a contra-indication	
Previous CAR T-cell therapy	Not applicable in trials Not in SPC	Not applicable in trials Not in SPC	Excluded	Not a contra-indication	Further CAR T therapy outside of clinical trials is to be avoided
History of autoimmune disease	Not an exclusion criterion	Not an exclusion criterion	Not an exclusion criterion	Not recommended in active autoimmune disease resulting in end-organ injury or requiring systemic immunosuppression or systemic disease-modifying agents within the last two years	Individualized risk-benefit assessment required
Current systemic immunosuppressive treatment	Any GVHD therapy must be stopped more than four weeks prior to enrolment to confirm that GVHD recurrence is not observed	Any immunosuppressive medication must be stopped more than four weeks prior to enrolment	Any immunosuppressive medication must be stopped more than four weeks prior to enrolment	Contra-indication	Intermittent topical, inhaled or intranasal corticosteroids are allowed
Existing or suspected fungal, bacterial, viral, or other infection	Active or latent hepatitis B or hepatitis C (test within eight weeks of screening) or any uncontrolled infection at screening	Uncontrolled active or latent hepatitis B or active hepatitis C; Uncontrolled acute life-threatening bacterial, viral or fungal infection (e.g. blood cultures positive <72 hours prior to screening)	Known history of HIV, hepatitis B (HepBs Ag positive) or hepatitis C (anti-HCV); Clinically significant active infection, or currently receiving IV antibiotics or within seven days of enrolment	Relative contra-indication; individualized risk-benefit assessment required	Active infection should be controlled and on treatment prior to leukapheresis
History of CNS disease	CNS involvement by malignancy defined as CNS-3 as per NCCN guidelines excluded; however, those with history of effectively treated CNS disease were eligible	Active CNS involvement by malignancy excluded	Subjects with detectable cerebrospinal fluid (CSF) malignant cells, or brain metastases, or with history of CSF malignant cells or brain metastases excluded	Relative contra-indication; individualized risk-benefit assessment required (19)	Caution required as higher risk of neurological toxicity

Table 1. Clinical trial patient selection eligibility criteria

Abbreviations. ALL: acute lymphoblastic leukaemia; DLBCL: diffuse large B-cell lymphoma; NHL: non-Hodgkin lymphoma; SPC: summary of product characteristics; ECOG: Eastern Cooperative Oncology Group; allo-HCT: allogeneic hematopoietic cell transplantation; GvHD: graft-versus-host disease; BiTE: bispecific monoclonal antibodies; CNS: central nervous system

Test methods	Trials and/or SPC	EBMT recommendations	Comment
Disease confirmation		Histology only for NHL Immunophenotyping for ALL	
Haematology			
Haematology	ANC>1.0x10 ⁹ /L in NHL trials	ANC > 1.0x10 ⁹ /L	Evidence of adequate bone marrow reserve
Chemistry			
Bilirubin	<26-34umol/L	<34umol/L; higher limit acceptable (<43umol/L) with Gilbert's syndrome	No trial data regarding patients outside of these parameters
AST/ALT	<5xULN	<5x ULN	Attempt to identify causes e.g. active infections
Creatinine clearance	Age- and gender-dependent cut-offs for ELIANA trial, > 60ml/min/1.73m ² (JULIET)	> 30 ml/min	Caution is required in patients with CrCl of <60ml/min
Virology			
Hepatitis B*	Active or latent hepatitis B (test within 8 weeks of screening) (ELIANA, JULIET)	Mandatory in some countries. To be done within 30 days of leukapheresis and results must be available at the time of collection and shipment	As per national guidelines Serology/molecular testing
Hepatitis C*	Active hepatitis C (test within 8 weeks of screening) (ELIANA, JULIET)	Mandatory in some countries. To be done within 30 days of leukapheresis and results must be available at the time of collection and shipment	As per national guidelines Serology/molecular testing
HIV*	HIV positive test within eight weeks of screening - ineligible for CAR T trials	Mandatory in some countries. To be done within 30 days of leukapheresis and results must be available at the time of collection and shipment	Kymriah™ is using a lentiviral vector whereas Yescarta™ uses a retroviral vector
Other Work-up			
Cardiac function	Hemodynamically stable and LVEF>45% confirmed by echocardiogram or MUGA scan; Patients with cardiac involvement by NHL were excluded from some trials	LVEF>40%; assess for pericardial effusion by echocardiography; ECG	Work-up of effusions required to identify causes
CNS imaging	ZUMA-1 trial required an MRI of the brain to confirm there was no evidence of lymphoma	MRI not required except in those with a history of CNS disease or current neurological symptoms of concern	A baseline MRI can be helpful, should severe neurological toxicities arise
Lumbar puncture	Patients with active CNS disease were excluded from trials	Lumbar puncture not required except in those with a history of CNS disease or current neurological symptoms of concern	
Fertility	Females of childbearing potential must have a negative serum or urine pregnancy test within 48 hours of infusion (ELIANA)	Females of childbearing potential must have a negative serum or urine pregnancy test	Test must be repeated and confirmed negative within eight days of the CAR-T cell infusion

Table 2. The minimum required tests

Abbreviations. SPC: summary of product characteristics; NHL: Non-Hodgkin Lymphoma; ALL: acute lymphoblastic leukaemia; ANC: absolute neutrophil count; ULN: upper limit of normal; LVEF: left ventricular ejection fraction; MUGA: multiple-gated acquisition; MRI: Magnetic resonance imaging; CNS: central nervous system. * Leukapheresis material for Kymriah™ manufacturing will not be accepted from patients with a positive test for active HBV, HCV or HIV (SPC)

Prior to Apheresis	Trials/SPC	EBMT recommendations	Comment
ECOG Performance status score	Not specified	ECOG ≤ 2	At discretion of apheresis practitioner
Days after last chemotherapy		Allow for recovery from cytotoxic chemotherapy	Need for marrow recovery from prior chemotherapy
Days off corticosteroids	Three (Kymriah TM) to seven (Yescarta TM) days off or on no more than prednisolone 5mg equivalent	Ideally, seven days to minimise effect on lymphocyte collection	A shorter period of as few as three days was considered acceptable by Kansagra <i>et al</i> (12) Physiological replacement doses of hydrocortisone permitted
Mandatory blood tests			
Hepatitis B, Hepatitis C, HIV, syphilis, and HTLV	Mandatory for all trials	Mandatory in some countries. To be done within 30 days of leukapheresis and results must be available at the time of collection and shipment	Only serological testing is required; nucleic acid testing (NAT) is not necessary if all serological testing is negative
Blood tests to ascertain suitability for apheresis			
C-reactive protein		Recommended to assess for ongoing infection	In patients with active infection, eligibility for apheresis will need to be decided on a case-by-case basis
Standard electrolytes and renal function		Required	Apheresis may predispose to electrolyte imbalance and limited fluid tolerance
Blood values required for optimal apheresis performance			
Haemoglobin		Haemoglobin > 80 g/L Haematocrit > 0.24	To establish a good interface during collection
Absolute neutrophil count (ANC)		$> 1.0 \times 10^9/L$	Consistent with recovery from prior chemotherapy
Absolute Lymphocyte count (ALC)		$> 0.2 \times 10^9/L^*$	Higher count required in small children. Of note, $0.2 \times 10^9/L$ CD3 ⁺ count is the minimum threshold
Platelet count		$> 30 \times 10^9/L$	Transfuse as required
Full Blood Count (FBC)		To be repeated at the end of apheresis procedure	Apheresis can remove more than 30% of circulating platelets

Table 3. Checklist prior to apheresis

Abbreviations. SPC: summary of product characteristics; ECOG: Eastern Cooperative Oncology Group.

* This threshold specifically applies to count recovery following corticosteroid therapy where an ALC > 0.2 is a surrogate marker of corticosteroid washout

Type of therapy	SPCs	EBMT recommendations	Comments
Allo-HCT	No guidance	Patients should be off immunosuppression and GVHD-free	A minimum of one month is recommended
DLI	No guidance	Four weeks	6-to-8 weeks may be safer to rule out any GVHD
High-dose chemotherapy	No guidance	3-to-4 weeks depending on the intensity of the chemotherapy	Recovery from cytopenias is required
CNS-directed therapy	No guidance	One week	
Short-acting cytotoxic/anti-proliferative drugs	No guidance	Three days	Recovery from cytopenias is required
Systemic corticosteroids	No guidance	Ideally, seven days to minimise any effect on lymphocyte collection	A shorter period of as few as three days was considered acceptable by Kansagra <i>et al</i> (12) Regardless of timing, an ALC > 0.2 x 10 ⁹ /L is preferable given the likely effect of recent corticosteroids on lymphocyte quality

Table 4. Wash-out period before leukapheresis (adapted from Kansagra et al, BBMT 2018) (12)

Abbreviations. SPC: summary of product characteristics; Allo-HCT: allogeneic hematopoietic cell transplantation; GVHD: graft versus host disease; DLI: donor lymphocyte infusion; ALC Absolute Lymphocyte Count

	SPCs	EBMT recommendations	Comments
CAR T-cell product	The availability of the CAR T-cell product must be confirmed prior to starting the LD conditioning	LD conditioning should only be administered following receipt of product on site	Exceptional situations may necessitate the administration of LD conditioning following confirmation of successful production but prior to arrival
Clinical conditions		Active infections must be excluded or under control before starting LD conditioning	Patient has to be able to tolerate LD conditioning
WBC	LD conditioning should be administered before the Kymriah™ infusion unless the WBC count within one week of the infusion is $\leq 1.0 \times 10^9/L$	Administer LD conditioning to all patients regardless of WBC or ALC	Some investigators have suggested that patients with low ALC ($< 0.1 \times 10^9/L$) may not require LD as these patients are already “lymphodepleted”

Table 5a. Checklist before starting the conditioning

Abbreviations. SPC: summary of product characteristics; LD: lymphodepletion; WBC: white blood cell count; ALC: absolute lymphocyte count

Test methods	Trials and SPC	EBMT recommendations	Comment
Chemistry			
C-reactive protein and/or fibrinogen level		Required to rule out ongoing infection	LD is contra-indicated in patients with active infection. Active infection must be excluded or under control before starting LD
Bilirubin	<26-34umol/L	<34umol/L; higher limit acceptable (>43umol/L) with Gilbert's syndrome	No trial data regarding patients outside of these parameters
AST/ALT	<5xULN	<5xULN	Attempt to identify causes e.g. active infections
Creatinine clearance		> 30 ml/min	Modify drugs doses according to Creatinine Clearance
Other Work-up			
Cardiac function		Repeat cardiac investigations only if clinically indicated (e.g. cardiotoxic bridging chemotherapy)	LVEF>40%; assess for pericardial effusion by echocardiography; ECG

Table 5b. Checklist of laboratory tests prior to conditioning

Abbreviations. SPC: summary of product characteristics; LD: lymphodepletion; ULN: upper limit of normal;

LVFE: left ventricular ejection fraction; ECG: electrocardiogram

	SPC	EBMT recommendations	Comment
Active infection	Reasons to delay treatment: active uncontrolled infection (Kymriah TM and Yescarta TM)	Contra-indication	CAR-T cell infusion should be delayed until the infection has been successfully treated or controlled
Cardiac arrhythmia not controlled with medical management	Reasons to delay treatment: unresolved SARs (esp. pulmonary reactions, cardiac reactions or hypotension) from preceding chemotherapies (Kymriah TM and Yescarta TM)	Cardiologist opinion is required	Specific individualized risk-benefit assessment required
Hypotension requiring vasopressor support	See above	Contra-indication	CAR-T cell infusion should be delayed until the hypotension has been fully treated
New-onset or worsening of another non-hematologic organ dysfunction \geq Grade 3		Work-up is needed to identify the cause	Specific individualized risk-benefit assessment required
Significant worsening of the clinical condition since start of LD	Reasons to delay treatment: significant clinical worsening of leukaemia burden or lymphoma following LD chemotherapy (Kymriah TM)	Work-up is needed to identify the cause	Specific individualized risk-benefit assessment required
Pre-medication	‘It is recommended that patients be pre-medicated with paracetamol and diphenhydramine or another H1 antihistamine within approximately 30 to 60 minutes prior to Kymriah TM infusion’ ‘Paracetamol given orally and diphenhydramine or chlorpheniramine or intravenous or oral (or equivalent) approximately 1 hour before Yescarta TM infusion is recommended’	As per SPC	
Concomitant medication	Corticosteroids should NOT be used prior to or around the time of the infusion except in case of a life-threatening emergency	As per SPC	

Table 6. Checklist and pre-medication before CAR-T cell infusion

Abbreviations. SPC: summary of product characteristics; LD: lymphodepletion

Period	SPCs and protocols	EBMT recommendations	Comments
Day 0 to Day +14 post-infusion	Some protocols require 5-14 days hospitalisation after the infusion	Ideally, 14 days hospitalisation	Shorter hospitalisation periods as well as outpatient follow-up are possible in centres that can provide 24/7 contact with immediate availability of specialist inpatient care. Patients have to be located within 30 minutes of the centre
From hospital discharge to Day +28 post-infusion	Some protocols require that patients be located within 30 to 60 minutes of the centre	<p>Patients have to be located within 60 minutes of the treating unit or a well-equipped centre*</p> <p>The continuous presence of a caregiver who is educated to recognize the signs and symptoms of CRS and ICANS is required</p>	CRS and, in particular, ICANS can occur after the patients has left the hospital. In addition, life-threatening complications may occur during this period e.g. septic shock in neutropenic patients

Table 7. Recommendations regarding the first month after CAR-T infusion

Abbreviations. SPC: summary of product characteristics; CRS: cytokine release syndrome; ICANS: effector cell-associated neurotoxicity syndrome. * Centres competent to manage such complications

Test	Points
Orientation: orientation to year, month, city, hospital	4
Naming: ability to name three objects (e.g. table, television, pillow)	3
Following commands: ability to follow simple commands (e.g. “smile” or “open your mouth”)	1
Writing: ability to write a standard sentence (e.g. “Happy to have my family around”)	1
Attention: ability to count backwards from 100 by 10	1

Table 8. ICE score for neurological toxicity assessment. Adapted from Lee *et al* (38)

	always	often	sometimes	rarely	never
Eye contact with caregiver	0	1	2	3	4
Purposeful actions	0	1	2	3	4
Aware of their surroundings	0	1	2	3	4
Being restless	4	3	2	1	0
Being inconsolable	4	3	2	1	0
Being underactive	4	3	2	1	0
slow response to interactions	4	3	2	1	0
Communicating needs and wants	4	3	2	1	0

Table 9. CAPD for encephalopathy assessment in children < 12 years

Adapted from Traube *et al* (61)

	Trials	EBMT recommendation	Comment
Neutropenia	G-CSF should be used according to published guidelines	G-CSF to shorten duration of neutropenia from 14 days post-infusion can be considered	Avoid if patient has CRS or ICANS There are theoretical concerns regarding macrophage activation
Antibacterial prophylaxis	Not recommended	Not recommended*	Can be considered in case of prolonged neutropenia and should be based on local guidelines e.g. with levofloxacin or ciprofloxacin
Anti-viral	Subjects should receive prophylaxis for infection with herpes virus, according to NCCN guidelines or standard institutional practice	Valaciclovir 500 mg bid or aciclovir 800mg bd	Start from LD conditioning until one year post-CAR T-cell infusion and/or until CD4 ⁺ count >0.2x10 ⁹ /L
Anti-pneumocystis	Subjects should receive prophylaxis for infection with pneumocystis pneumonia, according to NCCN guidelines or standard institutional practice	Co-trimoxazole 480 mg once daily or 960 mg three times each week To start from LD conditioning until one year post-CAR-T cell infusion and/or until CD4 ⁺ count >0.2x10 ⁹ /L	Can be started later depending on centre guidelines. In case of co-trimoxazole allergy, pentamidine inhalation (300 mg once every month), dapsone 100 mg daily or atovaquone 1500 mg once daily are other agents to consider
Systemic anti-fungal prophylaxis	Subjects should receive prophylaxis for infection with fungal infections according to NCCN guidelines or standard institutional practice	Not recommended routinely; however, consider in patient with prolonged neutropenia and on corticosteroids	In patients with prior allo-HCT, prior invasive aspergillosis and those receiving corticosteroids, posaconazole prophylaxis should be considered
IV Immunoglobulin	Gammaglobulin will be administered for hypogammaglobulinaemia according to institutional guidelines. At a minimum, trough IgG levels should be kept above 400 mg/dL, especially in the setting of infection	Routine in children, consider in adults who have had infections with encapsulated organisms	Clinical evidence does not support routine use in adults following allo-HCT

Table 10. Anti-infective prophylaxis after CAR T cell therapy

Abbreviations. G-CSF: granulocyte colony stimulating factor; CRS: cytokine release syndrome; LD: lymphodepleting conditioning; NCCN: The National Comprehensive Cancer Network, allo-HCT: allogeneic hematopoietic cell transplantation.

* In patients with neutropenic fever, empiric treatment with broad spectrum antibiotics is strongly recommended

Test	Purpose	Frequency	Comment
FBC, Biochemistry panel, LDH, Fibrinogen, CRP	Standard follow-up	At every visit and as clinically indicated	
CMV, EBV, Adenovirus	Viral reactivation	As clinically indicated	
Quantitative Immunoglobulins or Serum protein electrophoresis	Immune reconstitution	Monthly	Consider IV immunoglobulins
Peripheral blood Immunophenotyping – CD3/4/8/16+56/19+	Immune recovery	Once monthly for first three months, three monthly thereafter in first year	Guide to anti-infective prophylaxis
CAR T monitoring where kits are available for routine monitoring of anti-CD19 CAR T	CAR T persistence	Peripheral blood flow cytometry or transgene by molecular methods as clinically indicated	Not recommended by CAR T manufacturers

Table 11. Patient monitoring during the medium-term follow-up

Abbreviations. FBC: full blood count; CMV: cytomegalovirus; EBV; Epstein - Barr virus; IV: intravenous.

Post CAR-T	Stable patients	Complications	Disease monitoring	Comment
Day +100 to one year	Three-monthly	As clinically indicated	Frequency of visits required is disease-specific and could be performed by CAR T centre or referring clinician	Patients who proceed to subsequent allo-HCT, cytotoxic therapy and/or immune effector cell therapy should be followed as per Majhail <i>et al</i> 2012(75)
One year to fifteen years	Annually			

Table 12. Recommended minimum frequency of attendance at CAR-T centre for patients in remission for Late Effect monitoring

Abbreviations. Allo-HCT: allogeneic hematopoietic cell transplantation

Test	Purpose	Frequency	Comment
Full Blood Count, Biochemistry panel	Standard follow-up	At every visit	
Viral infection (PB PCR, NPA)	Viral reactivation	As clinically indicated	
Quantitative Immunoglobulins +/- Serum protein electrophoresis	Immune reconstitution	At every visit	
Peripheral blood Immunophenotyping – CD3/4/8/16+56/19*	Immune reconstitution	Every second visit	No longer required following normalisation
CAR T monitoring where kits are available for routine monitoring of anti-CD19 CAR T*	CAR-T persistence	Every visit. However, no longer required when absent for two consecutive tests	Testing for CAR T persistence is not standard. Checking for B cell depletion as a surrogate marker is an option
Endocrine function and other standard late effects testing appropriate to age	Standard follow-up	As clinically indicated	

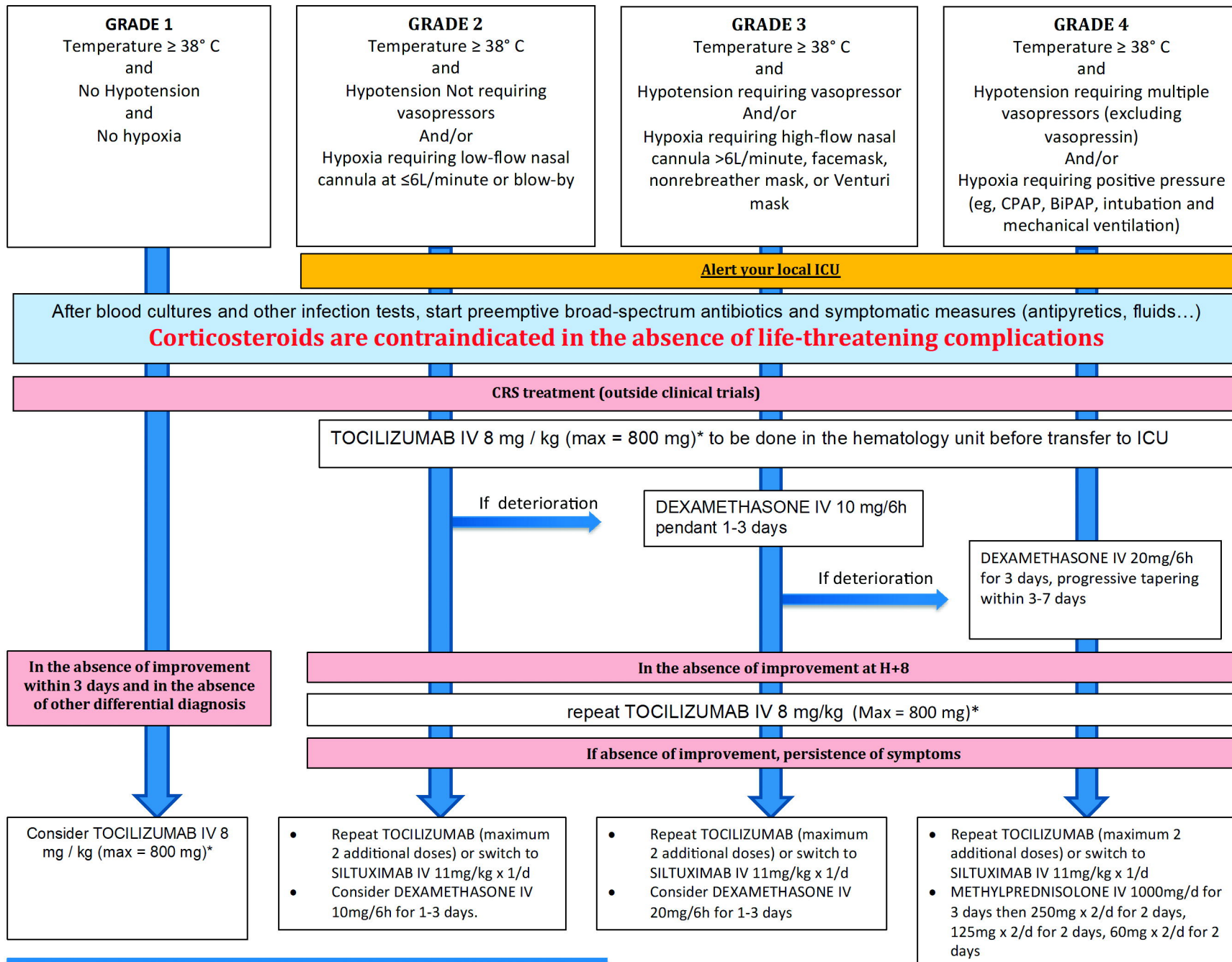
Table 13: Recommended tests to be performed at LTFU Clinic

Abbreviations: PB: peripheral blood; NPA: Naso-pharyngeal aspirate

*equivalent test methods for other immune effector cells as they become available

Figure 1. CRS management, adapted from Yakoub-Agha *et al* (56)

Figure 2. Management of CAR T-related neurological toxicity, adapted from Cornillon *et al* (62)



*In children less than 30 kg , TOCILIZUMAB is given at the dose of 12 mg/kg .

GRADE 1

- Decreased level of consciousness (Awakens spontaneously)
- ICE Score (age ≥ 12) = 7-9 or CAPD (age < 12) = 1-8

GRADE 2

- Alteration level of consciousness affecting activities of daily living (Awakens to voice)
- ICE Score (age ≥ 12) = 3-6 or CAPD (age < 12) = 1-8

GRADE 3

- Very altered level of consciousness (Awakens only to tactile stimulus)
- Any clinical seizure focal or generalized that resolves rapidly or non-convulsive seizures on EEG that resolve with intervention
- Focal/local oedema on neuroimaging
- ICE Score (age ≥ 12) = 0-2 or CAPD (age < 12) > 8

GRADE 4

- Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. 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- Cerebral oedema
- Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad
- ICE score or CAPD impossible

**Contact your local ICU,
Alert your referral neurologist**

Symptomatic measures: raised head 30 °, suspend oral nutrition, replace oral drugs by IV

Specific ICANS treatment (outside clinical trials)

- • Systematic EEG in 1st place
- • MRI and LP as clinically indicated (differential diagnosis)
- • Close monitoring

■ • Daily EEG, fundus, MRI and then LP in the absence of CI, transfer to ICU

- • If seizure (clinically or EEG): CLONAZEPAM IV 1 mg (0.015 mg / Kg up to 1 mg), and introduce levetiracetam 500 mg x2 (paediatric dose 30 mg / Kg x 2, max 3g daily)
- • If persistence or recurrence of seizure, repeat clonazepam 5 min once, otherwise, to be treated as a "état de mal"

- If papillary oedema : consider ACETAZOLAMIDE IV 1000 mg then 250-1000 mg/12h (5 mg/Kg/12h)

- If cerebral oedema: consider hyperosmolar therapy

If associated with CRS grade ≥ 1 (fever): TOCILIZUMAB IV 8 mg / kg (max = 800 mg); see management of CRS

If ICANS without CRS (afebrile): consider corticosteroid therapy

- DEXAMETHASONE IV 10 mg/6h for 1-3 d

- DEXAMETHASONE IV 20 mg/12h for 1-3 d

- • METHYLPREDNISOLONE IV 1000 mg/4h for 3 d then 250 mg x 2/d for 2 d, 125 mg x 2/d for 2 d, 60 mg x 2/d for 2 d
- • Discuss other alternative: high dose cyclophosphamide, anti-IL1R (Anakinra), antiIL6 (Siltuximab)

EEG monitoring until resolution: if seizure: e.g. ICANS grade 2
Daily fundus until resolution: if papillary oedema: e.g. ICANS grade 3
MRI and LP to be reassessed according to evolution
CAR-T cell checklist 2 times a day*

In children:
Grade 2-3: dexamethazone 0,5mg/kg /6h (maxi 10 mg/dose) ou methylprednisone 1 to 2 mg/kg/d.
Grade 4 : High-dose

Supplement 1: How to perform leukapheresis

Local apheresis experience should be used to benchmark apheresis outcomes (1-3), most importantly, Collection Efficiency (CE) (4).

CE for T-cells and similarly for total MNCs is calculated using the formula:

$$\text{CE} = \text{T-cells in bag} / (\text{peripheral blood T-cells per Litre} \times \text{processed blood volume in Litres}) \times 100\%$$

Thus, in a normal DLI donor with a peripheral blood T cell count of $2 \times 10^9/\text{L}$ at the onset of the apheresis and $10 \times 10^9/\text{L}$ T cells in the bag after an 8 Litre apheresis, the CE is calculated as follows:

$$\text{CE} = 10 \times 10^9 / (2 \times 10^9 \times 8) \times 100\% = 10/16 \times 100\% = 62.5\%$$

CE is then used to estimate the volume that will need to be processed to achieve the target dose of T-cells. For those manufacturers indicating target doses for mononuclear cells, CE can be calculated for MNC using this method and target volumes gauged accordingly (see below). However, not all commercial CAR T-cell manufacturers provide target cell counts for the apheresis product; some instead request the processing of a certain Blood Volume, regardless of patient size and lymphocyte counts. However, a dose of one-to-two billion T-cells is usually sufficient to start CAR T-cell manufacturing. CE and a target number allow for the calculation of the blood volume that needs to be processed in order to achieve this target. The formula to calculate target process volume is as follows:

$$\text{Process Volume (Liters)} = \text{T-cell target dose} / (\text{CE} \times \text{T-cell concentration in blood}) \text{ (Liters)}$$

As an example, a typical patient undergoing apheresis might have a peripheral blood CD3+ count of 200/ μ L; in this case, the target process volume is calculated as follows:

$$PV = 10^9 / ((0.4 \times 200 \times 10^6) / \text{Liters}) = 1000 / 80 \text{ Liters} = 12.5 \text{ Liters}.$$

Although the CE of 62.5% used in the calculation of our first example is a fairly typical CE, significant inter-individual variation between donors and recipients necessitates working with a significant margin of error. We therefore recommend working with a Collection Efficiency which at least 90% of patients have achieved, based on local experience. The 40% CE used in the second example is based on this principle. Benchmarking one's own apheresis performance is recommended. Typically, apheresis is relatively more efficient at lower leukocyte counts and the calculated CE will deteriorate the longer the patient is processed. In adults this will rarely be relevant but it may be a factor in small children.

In light of these factors, the collection of an adequate cell count in a smaller patient in whom less blood can be processed requires a correspondingly higher peripheral blood lymphocyte count. For normal-sized adults, a peripheral blood CD3⁺ cell count of 200/ μ L will usually suffice to achieve reasonable cell doses in the apheresis product. Currently, most commercial and clinical protocols do not contain strict guidance as to minimal lymphocyte counts, and apheresis targets are not always defined as a specific cell number in the bag. For patients with very low lymphocyte counts, more than one apheresis may be necessary to achieve the target dose although in adults this will be an infrequent occurrence.

Performing apheresis collection

Two large-bore venous access lines supporting adequate blood flow are required for leukapheresis. Fresh lines are preferable to long-standing catheters due to the risk of bacterial contamination. For adults, adolescents and children weighing more than 15 kg, peripheral venous access usually suffices; in low-weight children as well as very occasionally in adults, the placement of a central line may be necessary. If so, this should be formally scheduled to take place in advance of the planned time for starting apheresis, especially for Marketing Authorization Holders which require fresh apheresis material as the courier in charge of transporting the collected cell product may otherwise be delayed. Prior to apheresis, patient identity is confirmed using standard local procedures and the apheresis bag is labelled in accordance with local and MAH requirements. The specific patient identifiers required by a given CAR T-cell manufacturer may vary; however, the use of unique patient identifiers is critical as no further identity checks (e.g. HLA typing) will be performed during manufacturing or before re-infusion and it is critical that the chain of custody/chain of identity is maintained throughout the multi-stage manufacturing process until final administration to the patient.

Anti-coagulation is initially achieved with ACD-A at a 1:10-1:12 ratio though this may be reduced over time. Most manufacturers discourage additional use of heparin as it may interfere with down-stream processing. The amount of ACD-A allowed per minute and hence, inlet flow, is limited by the patient's total blood volume. Veins permitting, higher flow can be achieved by raising the infusion rate of ACD-A; this predisposes patients to significant electrolyte shifts which should be monitored regularly and, if necessary, corrected with i.v. or oral electrolytes (mostly calcium and potassium). The apheresis collection should target a "light" colour with a final Hb concentration of 4 g/dL or less. Typical low MNC counts, as

seen in patients, allow for the reduction of collection flow rates, thus limiting product size and plasma depletion of the patient. At the end of the apheresis procedure, labelling of the apheresis bag is completed prior to its separation from the apheresis set using sterile tube welding devices; clamps are no longer acceptable. Some MAH have specific requirements regarding the length of tubing that needs to be left attached to the bag; in addition, some ask that the tubing not be stripped. Apheresis data should be recorded according to local practice, including, as a minimum, apheresis start and end times as well as product volume.

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