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THAWING AND INFUSION OF CAR-T CELL PRODUCTS

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ABSTRACT

Some patients with hematologic malignancies may benefit from chimeric antigen receptor-T (CAR-T) cell treatment. Usually, the manufacture of this cells is carried out by centralized and specialized cell therapy laboratories that cryopreserve the CAR-T cells in bags and send it for infusion in the patient treatment center. In this context, the thawing and infusion of the CAR-T cell products are critical steps in this custody chain. This article aims to discuss the most relevant points related with the CAR-T cell thawing and infusion and proposes a kind of standard operational protocol (SOP) for these processes.

Keywords: CAR-T cells; thawing; infusion.

OBJECTIVES

Describe steps in the process of thawing CAR-T cell products, avoiding damage of product and loss of viability or sample contamination.

Describe check list steps before its infusion for correct administration.

Describe pre-infusion measures, including premedication use.

INTRODUCTION

The thawing and infusion procedure are critical steps to ensure the safety and effectiveness of the chimeric antigen receptor-T (CAR-T) cell treatment. The background with progenitor cell infusion provides a basis for infusion of other products such as CAR-T cells. Even though they are similar procedures, a validation process is suggested for each product, determining correct handling of them. General guidelines for handling CAR-T cells products must be established at each institution. Products manufactured by industry have specific guidelines and must be included in operational procedures. Procedures and measures adopted during thawing and infusion contribute to prevent, identify and minimize possible complications.

CAR-T cells can be fresh infused or cryopreserved.[1,2] Advantages of fresh product are associated with lower toxicity, due to the absence of cryoprotectant solution, but it requires infusion without conclusion of quality control tests. Cryopreservation allows conclusion of all quality controls prior to infusion, expands availability of product to centers far from manufacturing, but requires an infrastructure for storage until its administration.[1]

Fresh products manufactured in another institution must be transported refrigerated, with a temperature between 2-8ºC and cryopreserved products must be transported at temperatures below minus140ºC.[1] Transport of genetically modified organisms must follow national rules according to risk assessment of each vector used to manufacture CAR-T cells and procedures and measures to protect environmental and human health.[3]

Indication and contraindication

All patients in a CAR-T cells treatment protocol, with fresh-infused or cryopreserved products must follow recommendations, with no contraindication for this protocol.

Minimum requirements:

- Initial training of all employees involved in the process of thawing and infusion of CAR-T cells. The annual competence of all staff is required.
- Product release report approved by the Director of Cell Therapy Lab and the quality manager.
- In case of exceptional release – report and release approved by Medical Director of Cell Therapy Lab.
- Equipment for thawing products calibrated and cleaned with 70º alcohol prior to use.
- Transport container validated.
- Medical prescription for the infusion.

Material:

- Blood transfusion set
- Infusion set Y-Type, dual spike (optional)
- Sterile plastic bag or zip lock
- Saline in a 50 or 100 mL bag
- Water bath at 37ºC with sterile water or saline solution or dry bath
- Transport container (dry shipper or suitcase)

Pre and post-procedure guidance:

- Prior to procedure, medical prescription for infusion should be checked and premedication administered.
- Carry out a double-check of product with the nurse team, ensuring that correct product will be administered to correct patient.
- Perform pre-infusion care checks.
- Educate patient about the infusion process and possible post-infusion adverse effects.
- Perform vital signs control periodically during infusion.

PROCEDURE

Product receipt:

All centers must adopt strategies to check the integrity of the product, its identification and for products manufactured in other institutions, the transportation conditions, as described in Annex I. The centers must have facilities and equipment for storage of genetically manipulated products according to their risk assessment. It is important to check the bags and cassettes size and configuration prior their receipt and verify if they will fit in the nitrogen tank rack.[4]

The process to check and transfer cryopreserved units in storage tank must be carried out carefully and quickly, thus avoiding heating of products and the possibility of reaching critical temperatures, which can lead to loss of viability.[5,6]

Patient evaluation before infusion:

Before infusion, patient should be assessed. Changes in his clinical condition may postpone product infusion, as described in table 1.

TABLE 1 - Clinical conditions and its recommendations before CAR-T cell infusion. Adapted.[4]

Clinical condition	Recommendation	Comments
Active infection	Contraindication for infusion	Infection must be controlled or treated prior to infusion
Cardiac arrhythmia	Specialist release	Individual risk-benefit assessment
Hypotension requiring vasopressor	Contraindication for infusion	Infusion should be delayed until complete resolution of hypotension
Non-hematological clinical worsening with new comorbidity or worsening of previous comorbidity	Identify cause	Individual risk-benefit assessment
Disease progression	Identify cause	Individual risk-benefit assessment

Previous use of corticosteroids may impair the effectiveness of treatment, and its use is suggested only in life-threatening cases. Some clinical studies also advise washout intervals for drugs that could affect the cells therapeutic effect or be associated with increased risk to the patient, such as monoclonal antibody, antiproliferative therapy including oral and intrathecal chemotherapy, as described in table 2. Generally, recommended washout times are 2 weeks for systemic chemotherapy, 4 weeks for pegylated l-asparaginase, and 72 hours for steroids.[7] A check list should be applied to guarantee that all clinical conditions were evaluated before infusion, as described in Annex I.

TABLE 2 – Washout drugs before CAR-T infusion

Type of therapy	Recommendations
Corticosteroid	Stop steroids therapeutic dose 3 days prior to infusion[7,8]
Chemotherapy (including low dose maintenance therapy)	Stop ≥ 2 weeks before infusion[7,8]
Short-acting drugs used to treat leukemia or Lymphoma (hydroxyurea, tyrosine kinase inhibitors [TKI])	TKIs and hydroxyurea must be stopped ≥ 3 days before infusion[8]
Immunomodulatory drugs	Stop antibodies 4 weeks before infusion[8]
Prior radiation therapy	Radiation must be completed > 2 weeks before CAR-T infusion[8]
Intrathecal chemotherapy	Central nervous system prophylaxis must be stopped ≥1 week before[8]
Graft-vs-host disease therapies (e.g. calcineurin inhibitors)	Stop > 2 weeks before CAR-T infusion to confirm that GvHD recurrence is not observed[9]
Immunosuppressive therapy	Stop ≥ 2 weeks before infusion[8]

Thawing CAR-T cells product:

Before thawing product, a double check must be performed by trained employee to verify identifiers and their match in all documentation. Identifiers include patient name, record number or date of birth.

Although bone marrow transplant team is used to thaw progenitor cells, some precautions are important to avoid bacterial contamination and ensure the best viability.

Water baths are devices commonly used, although literature shows dry equipment as an alternatives in this process, without impacting viability and reducing risks of contamination.[4,10,11]

Temperature for thawing should be programmed to 37ºC (± 2ºC) and must be checked before thawing each aliquot. Thawing product in higher temperatures will impair its viability. The use of a secondary plastic bag during thawing is suggested to facilitate the cell recuperation in case of bag rupture and to reduce the risk of product bacterial contamination.[4]

After thawing, the product must be immediately infused as the cryoprotectant solution can damage the cells at room temperature. Thus, it is suggested to conduct thawing procedure quickly, homogenizing sample, to avoid temperature gradients in product and remove it from the thawing device once the process is completed. If more than one bag will be infused, wait to thaw next bag until it is determined that previous bag has been safely infused.

Steps for thawing product:

- Make sure water bath or dry thaw equipment is cleaned with germicidal wipes and sterile alcohol.

- Fill it with sterile water, according to institutional procedure.

- Make sure the water bath has been turned on and is ready for thawing the CAR-T cells infusion bag in advance.

- Heat the water bath at least 30 minutes before the thaw start time to allow the equipment to reach the temperature of 37°C.

- Remove the CAR-T cells product from the transport container using cryogloves.

- Remove the CAR-T cells infusion bag from its cassette.

- Examine the infusion bag for breaks or cracks

- Place the infusion bag in a secondary bag to avoid direct contact between the bag and the water.

- Immerse the bag in water bath and homogenize the product with care.

- Remove the bag from the water bath as soon as the process is completed.

Product infusion

Premedication

To prevent acute adverse effects of T cell infusions patient should be premedicated with antipyretic

and diphenhydramine or another H1 antihistamine approximately 30 to 60 minutes before the infusion. These medications should be repeated every 6 hours as needed.[4,7]

Infusion

A transfusion set is required for cell infusion. It is important to emphasize that infusion fluids set, bacterial filter or transfusion set with leukocyte depletion filter are unacceptable. Some manufacturers recommend using unfiltered tubing set, however Brazilian standards for infusion device require macroaggregate filter in all sets.[4,12]

The line to use for CAR-T cells infusion must be clearly designated. No simultaneous drugs can be administered in this line during the infusion.

Infusion should start as soon as product has been thawed, usually no longer than 30 minutes.

Dimethyl sulfoxide, used as a cryoprotectant, can be toxic to progenitor cell products. Although some studies with mononuclear cells show less toxicity, it seems prudent to infuse it as soon as possible after thawing.[13] CAR-T cells product has small volumes that allow rapid infusion.

Transfusion set should be primed using 0.9% sodium chloride before CAR-T cells bag is connected to device. At the end of infusion, transfusion set line should be washed with saline. In Y-type set, it is possible to wash the CAR-T cells bag with saline connected on other spike, before washing line.

Infusion bag and set must be discarded in accordance with specific institutional policies and regulations of genetically modified organism waste.

CRITICAL POINTS AND RISKS

For early detection of adverse event, vital signs should be monitored prior to, during and immediately after infusion, then every 15min for the 1st hour and every 30min for 2nd hours or until the signs are satisfactory and stable.

Prior to infusion, 2 doses of tocilizumab must be confirmed as available for the management of cytokine release syndrome and its related adverse events.

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ANNEX

ANNEX I - Receipt of CAR-T product

Patient identification	
Name:	Medical record number:
Date of birth:	Protocol Nº:

Affix patient identification tag

Product Identification:			
Donor identification number (DIN):			
Product:			
( ) HPC(CBU)	( ) HPC, APHERESIS	( ) HPC, MARROW	( ) MNC, APHERESIS
( ) HPC, CD34 APHERISIS Enriched	( ) MSC, MARROW	( ) CAR-T	( ) _____ _____
Number of bags:		Number of tubes:	

DOCUMENTS:	
Product report ( ) yes ( ) no	( ) yes ( ) no
Release and product report	( ) yes ( ) no
Signature and date:	
PRODUCT TRANSPORT AND PACKAGING	
Box/dry shipper	( ) yes ( ) no
Box/dry shipper sealed	( ) yes – seal number_____ ( ) no
Temperature	_____ºC
Transport time	_____ hours and _____ minutes
Signature and date and hour:	

PRODUCT AND SAMPLES	
Correct bag identification	( ) yes ( ) no
Patient ID is correct	( ) yes ( ) no
All tubes are identified with the same product number	( ) yes ( ) no
Time to storage product (from box/dewar until its storage in nitrogen tank):	_____ min (cryopreserved product should be storage in 2 minutes)
Integrity	( ) yes ( ) no
Visual inspection	( ) Ok ( ) no _____
Comments: ( ) not applicable	
Signature and date:	Signature and date:

ANNEX II – Patient assessment for CAR-T infusion

Patient identification	
Name:	Medical record number:
Date of birth:	Protocol Nº:

Affix patient identification tag

Clinical Condition	
Signs of active infection last 48 hours	( ) yes - contraindication to administer ( ) no
Cardiac arrhythmia	( ) yes – specialist assessment: ( ) Released by _____ ( ) Not released ( ) no
Hypotension requiring vasopressor	( ) yes - contraindication to administer ( ) no
Clinical worsening	( ) yes – assessment: ( ) Released by _____ ( ) Not released ( ) no
Disease progression	( ) yes – assessment: ( ) Released by _____ ( ) Not released ( ) no
Drugs	
( ) Corticosteroid: _____ dose_____ last dose date _____ ( ) released by _____ ( ) not released	
( ) monoclonal antibody: _____ dose_____ date last dose_____ ( ) released by _____ ( ) not released	
( ) chemotherapy: _____ dose_____ date last dose_____ ( ) released by _____ ( ) not released	
( ) Radiotherapy: _____ dose_____ date last dose_____ ( ) released by _____ ( ) not released	