Management of adults and children undergoing chimeric antigen receptor 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)

Ibrahim Yakoub-Agha,[1] Christian Chabannon,[2] Peter Bader,[3] Grzegorz W. Basak,[4] Halvard Bonig,[5] Fabio Ciceri,[6] Selim Corbacioglu,[7] Rafael F. Duarte,[8] Hermann Einsele,[9] Michael Hudecek,[9] Marie José Kersten,[10] Ulrike Köhl,[11] Jürgen Kuball,[12] Stephan Mielke,[13] Mohamad Mohty,[14] John Murray,[15] Arnon Nagler,[16] Stephen Robinson,[17] Riccardo Saccardi,[18] Fermin Sanchez-Guijo,[19] John A. Snowden,[20] Micha Srour,[21] Jan Styczynski,[22] Alvaro Urbano-Ispizua,[23] Patrick J. Hayden[24] and Nicolaus Kröger[25]

[1]CHU de Lille, LIRIC, INSERM U995, Université de Lille, Lille, France; [2]Institut Paoli-Calmettes & Module Biothérapies, INSERM CBT-1409, Centre d'Investigations Cliniques de Marseille, Marseille, France; [3]Clinic for Children and Adolescents, University Children's Hospital, Frankfurt, Germany; [4]Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland; [5]Institute for Transfusion Medicine and Immunohematology of Goethe University and German Red Cross Blood Service, Frankfurt, Germany; [6]Università Vita-Salute San Raffaele, IRCCS Ospedale San Raffaele, Milan, Italy; [7]Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, University Hospital of Regensburg, Regensburg, Germany; [8]Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain; [9]Medizinische Klinikund Poliklinik II, Universitätsklinikum Würzburg, Würzburg, Germany; [10]Department of Hematology, Amsterdam UMC, University of Amsterdam, Cancer Center Amsterdam and LYMMCARE, Amsterdam, the Netherlands; [11]Fraunhofer Institute for Cellular Therapeutics and Immunology (IZI) and Institute of Clinical Immunology, University of Leipzig, Leipzig as well as Institute for Cellular Therapeutics, Hannover Medical School, Hannover, Germany; [12]Department of Hematology and Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, the Netherlands; [13]Department of Laboratory Medicine/Department of Cell Therapy and Allogeneic Stem Cell Transplantation (CAST), Karolinska Institutet and University Hospital, Stockholm, Sweden; [14]Hôpital Saint-Antoine, AP-HP, Sorbonne Université, INSERM UMRS 938, Paris, France; [15]Christie Hospital NHS Trust, Manchester, UK; [16]The Chaim Sheba Medical Center, Tel-Hashomer, Affiliated with the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel; [17]University Hospitals Bristol NHS Foundation Trust, Bristol, UK; [18]Hematology Department, Careggi University Hospital, Florence, Italy; [19]IBSAL-Hospital Universitario de Salamanca, CIC, Universidad de Salamanca, Salamanca, Spain; [20]Department of Haematology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK; [21]Service des Maladies du Sang, CHU de Lille, Lille, France; [22]Department of Pediatric Hematology and Oncology, Collegium Medicum, Nicolaus Copernicus University Torun, Bydgoszcz, Poland; [23]Department of Hematology, ICMHO, Hospital Clínic de Barcelona, Barcelona, Spain; [24]Department. of Hematology, Trinity College Dublin, St. James's Hospital, Dublin, Ireland and [25]Department of Stem Cell Transplantation, University Medical Center Hamburg, Hamburg, Germany

 doi:10.3324/haematol.2019.229781

Received: June 16, 2019.

Accepted: November 19, 2019.

Pre-published: November 21, 2019.

Correspondence: *PATRICK J. HAYDEN* -phayden@stjames.ie

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:

CE = T-cells in bag/ (peripheral blood T-cells per Litre x processed blood volume in Litres) x 100%

Thus, in a normal DLI donor with a peripheral blood T cell count of 2 $x10^9$/L at the onset of the apheresis and $10x10^9$/L T cells in the bag after an 8 Litre apheresis, the CE is calculated as follows:

CE = $10x10^9$/ ($2x10^9$ x 8) x 100% = 10/16 x 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:

Process Volume (Liters) = T-cell target dose/ (CE x T-cell concentration in blood) (Liters)

As an example, a typical patient undergoing apheresis might have a peripheral blood CD3+ count of 200/μL; 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/μL 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.

References

1. Allen ES, Stroncek DF, Ren J, Eder AF, West KA, Fry TJ, et al. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. Transfusion. 2017 May;57(5):1133-41.

2. Bersenev A. CAR-T cell manufacturing: time to put it in gear. Transfusion. 2017 May;57(5):1104-6.

3. Even-Or E, Di Mola M, Ali M, Courtney S, McDougall E, Alexander S, et al. Optimizing autologous nonmobilized mononuclear cell collections for cellular therapy in pediatric patients with high-risk leukemia. Transfusion. 2017 Jun;57(6):1536-42.

4. Ceppi F, Rivers J, Annesley C, Pinto N, Park JR, Lindgren C, et al. Lymphocyte apheresis for chimeric antigen receptor T-cell manufacturing in children and young adults with leukemia and neuroblastoma. Transfusion. 2018 Jun;58(6):1414-20.