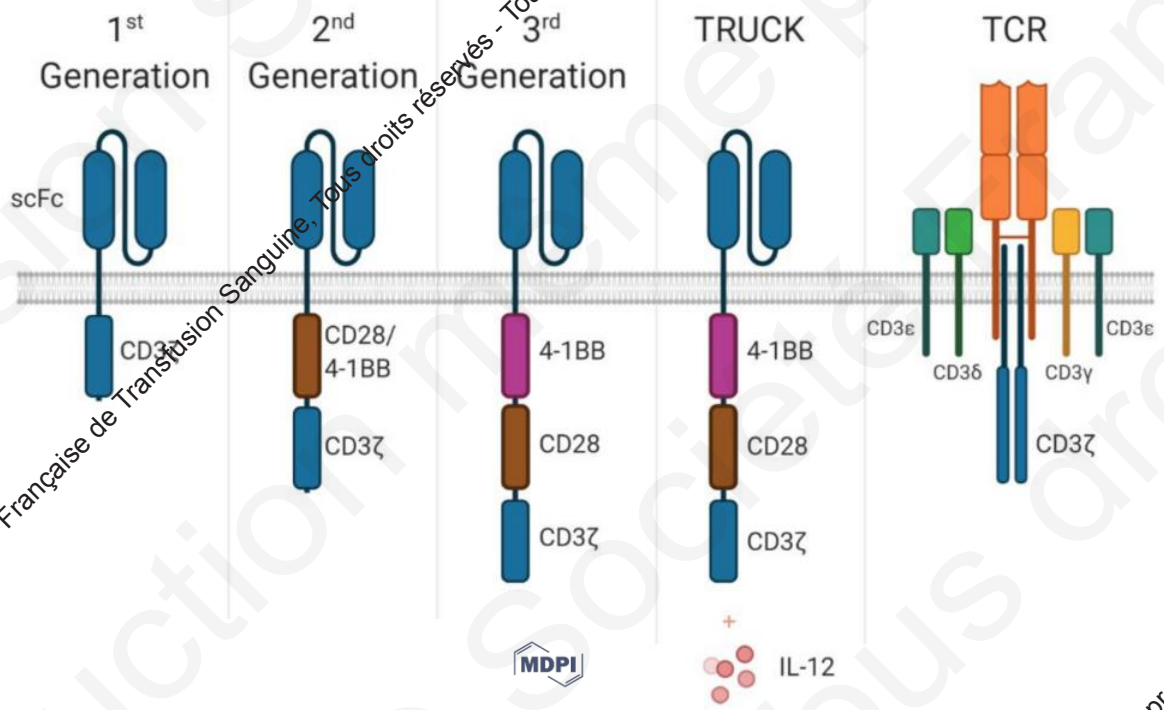
CRCM
Centre de Recherche en Cancérologie de Marseille

Production de CAR-T Cells fabriqués industriellement : contribution des établissements de transfusion et des hôpitaux à la mise à disposition de la matière première biologique

Pr Christian Chabannon
Professeur de Biologie Cellulaire. Faculté des Sciences Médicales & Paramédicales. Aix-Marseille Université.
Chef de Service & Personne Responsable. Centre de Thérapie Cellulaire. Institut Paoli-Calmettes. Marseille
Chair. EBMT Cellular Therapy & Immunobiology Working Party

DISCLOSURES OF COMMERCIAL SUPPORT

Name of Company	Research support	Employee	Consultant	Stockholder	Speaker's Bureau	Scientific Advisory Board	Other
Sanofi SA	X		X				X
Bellicum Pharmaceuticals			X			X	
Kite / Gilead			X			X	X
BMS / Celgene			X			X	X
Novartis			X				X
Janssen			X			X	
Terumo BCT			X				X



cells

MDPI

Review

T-Cell Gene Therapy in Cancer Immunotherapy: Why It Is No Longer Just CARs on The Road

Michael D. Crowther [1,*], Inge Marie Svane [1] and Özcan Met [1,2,*]

[1] Department of Oncology, National Center for Cancer Immune Therapy (CCIT-DK), Copenhagen University Hospital Herlev, 2730 Herlev, Denmark; Inge.Marie.Svane@regionh.dk

[2] Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

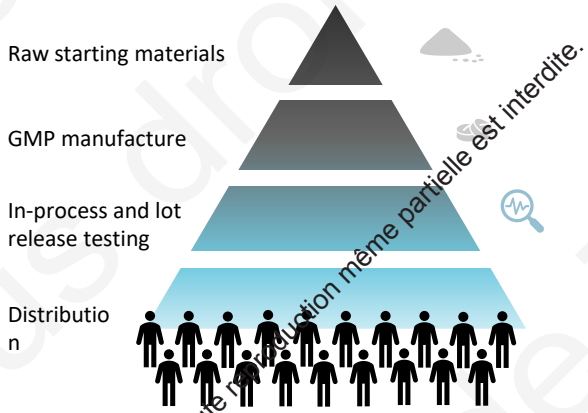
* Correspondence: michael.douglas.crowther@regionh.dk (M.D.C.); Ozcan.Met@regionh.dk (Ö.M.)

CAR T cell manufacturing process differs of the production of conventional drug products

CAR T cell manufacturing process:

- Personalised to each patient[1]
- Inherent variability of starting product[1]
- Limited batch size[2]
- Complex manufacturing process[1,2]
- Specialised facility with highly trained personnel[3]
- OOS production[1]

Conventional manufacture[4]



CAR: chimeric antigen receptor; GMP: good manufacturing practice; OOS: out of specification

1. Bersenev A & Kili S. *Cell Gene Ther Insights* 2018; 4:1051–1058. 2. Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products (Jun 2017; available at https://ec.europa.eu/health/sites/health/files/files/eudralex/vol4/2017_11_22_guidelines_gmp_for_atmps.pdf). 3. Yescarta SmPC (May 2019; available at www.ema.europa.eu). 4. Adapted from Scientific and Regulatory Considerations for Gene Modified T Cell Therapy. (Nov 2017; available at https://pharm.ucsf.edu/sites/pharm.ucsf.edu/files/cersi/media-browser/Graeme%20Price%20and%20Kristin%20Baird.pdf).

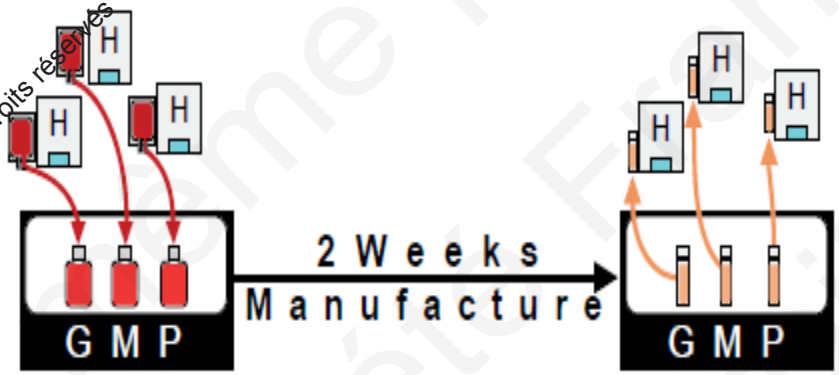


Figure 1. Centralized CD19 CAR T Cell Manufacturing

CD19 CAR T cell products are manufactured centrally from shipped patient apheresis units. Clinical grade products are then cryo-shipped back to the respective patients' institutions for infusion.

CAR-T Cells approved indications for hematological malignancies

CID	MAH	Date of MA in Europe	Indications	Targeted tumor antigen
tisagenlecleucel	Novartis	August 2018	r/r ALL (< 26 years old) r/r NHL	CD19
axicabtagene ciloleucel	Kite / Gilead	August 2018	r/r NHL & PMBCL	CD19
brexucabtagene autoleucel	Kite / Gilead	December 2020	r/r MCL r/r ALL	CD19
lisocabtagene maraleucel	Celgene / BMS	not yet approved	r/r NHL	CD19
idecabtagene vicleucel	Celgene / BMS	August 2021	adv Multiple myeloma	BCMA
ciltacabtagene autoleucel	Legend Pharm. / Janssen Pharm. / J&J	not yet approved	adv Multiple myeloma	BCMA

ICD (commercial name)	MAH	Indication(s)	Date MA was granted	Vector type	Co-stimulation domain	Registration studies	Real-World Data (RWD)	ORR	CRS / ICANS incidence (all grades)
tisagenlecleucel	Novartis	r/r B-cell ALL *	August 2017 (FDA) August 2018 (EMA)	lentiviral	4-1BB	ELIANA Maude SL et al. N Engl J Med. 2018 Feb 1;378(5):439-448. doi: 10.1056/NEJMoa1709866	Pasquini MC et al. Blood Adv. 2020 Nov 10;4(21):5414-5424. doi: 10.1182/bloodadvances.2020003092.	81%	77% / 40%
		r/r DLBCL	October 2017 (FDA) August 2018 (EMA)			JULIET Schster SJ et al. N Engl J Med. 2017 Dec 28;377(26):2545-2554. doi: 10.1056/NEJMoa1708566. Epub 2017 Dec 10	Iacoboni G et al. Cancer Med. 2021 May;10(10):3214-3223. doi: 10.1002/cam4.3881. Epub 2021 May 1.	52%	58% / 21%
axicabtagene ciloleucel	Kite / Gilead	r/r DLBCL r/r PMBCL	October 2017 (FDA) August 2018 (EMA)	retroviral	CD28	ZUMA-01 Neelapu SS et al. N Engl J Med. 2017 Dec 28;377(26):2531-2544. doi: 10.1056/NEJMoa1707447. Epub 2017 Dec 10.	Jacobson CA et al. J Clin Oncol. 2020 Sep 20;38(27):3095-3106. doi: 10.1200/JCO.19.02103. Epub 2020 Jul 15	82%	93% / 64%
relmacabtagene autoleucel	JW Therapeutics	r/r DLBCL	September 2021 (National Medical Products Administration (NMPA) of China)			RELIANCE	Zhu J, et al. ASH 2020. Abstracts 1186		

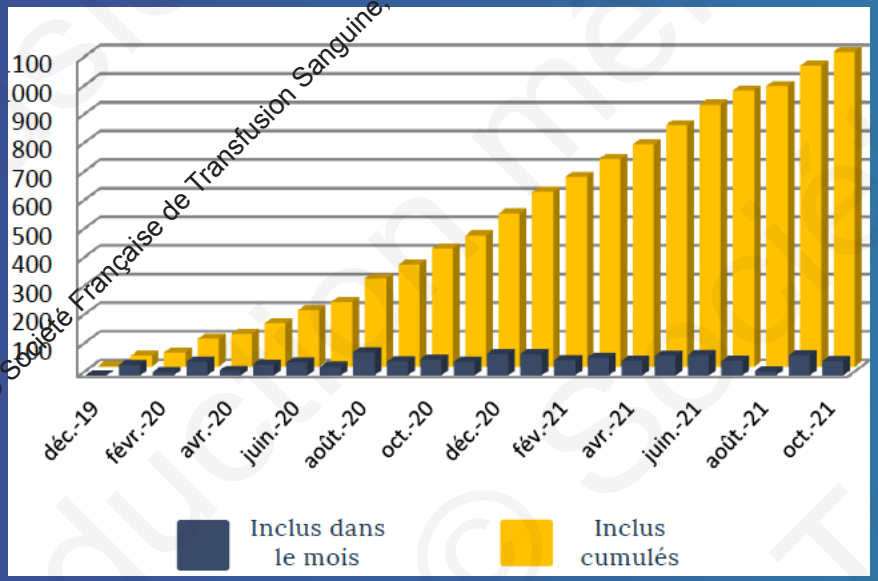
Inter-trial comparisons should not be made due to differences in study design, patient populations, treatment interventions, and duration of follow-up, among others. We cannot make direct comparisons or draw conclusions from one trial to another. For descriptive purposes, efficacy results for each of the studies mentioned are listed.

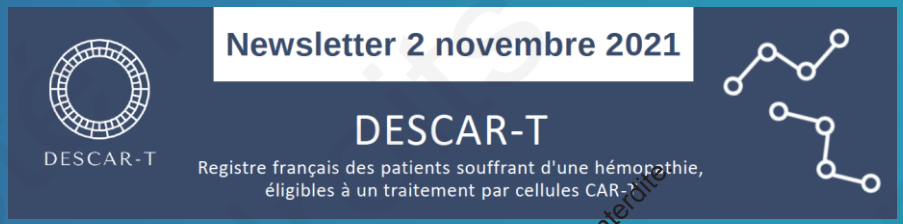
Chabannon C et al. Bull Cancer. 2021. in press

ICD (commercial name)	MAH	Indication(s)	Date MA was granted	Vector type	Co-stimulation domain	Registration studies	Real-World Data (RWD)	ORR	CRS / ICANS incidence (all grades)
brexucabtagene autoleucel	Kite / Gilead	r/r MCL	July 2020 (FDA) December 2020 (EMA)	retroviral	CD28	ZUMA-02 Wang M et al. N Engl J Med. 2020 Apr 2;382(14):1331-1342. doi: 10.1056/NEJMoa1914347	NA	85%	91% / 63%
lisocabtagene maraleucel	BMS (Celgene)	r/r DLBCL	February 2021 (FDA)	lentiviral	4-1BB	TRANSCEND Abramson JS et al. Lancet. 2020 Sep 19;396(10254):839-852. doi: 10.1016/S0140-6736(20)31366-0. Epub 2020 Sep 1	NA	73%	42% / 30%
idecabtagene vicleucel	BMS (Celgene)	MM progression after treatment with one agent from the three major classes	March 2021 (FDA) / August 2021 (EMA)	lentiviral	4-1BB	KARMMA Munshi NC et al. N Engl J Med. 2021 Feb 25;384(8):705-716. doi: 10.1056/NEJMoa2024850	NA	73%	84% / 18%
ciltacabtagene autoleucel	Janssen (J&J / Legend Therapeutics)	MM progression after treatment with one agent from the three major classes		lentiviral	4-1BB	CARTITUDE Berdeja JG et al. Lancet. 2021 Jul 24;398(10297):314-324. doi: 10.1016/S0140-6736(21)00933-8. Epub 2021 Jun 24	NA	97%	95% / 21%

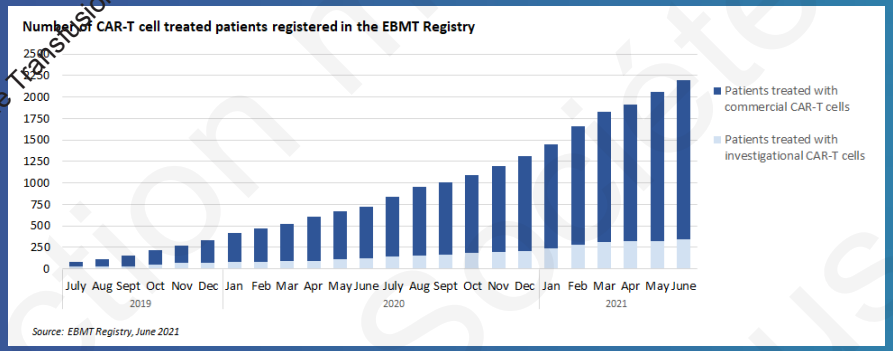
Inter-trial comparisons should not be made due to differences in study design, patient populations, treatment interventions, and duration of follow-up, among others. We cannot make direct comparisons or draw conclusions from one trial to another. For descriptive purposes, efficacy results for each of the studies mentioned are listed.
US FDA has approved liso-cel for the treatment of r/r DLBCL . Liso-cel is not approved by EMA in Europe.
Cilta-cel is not approved by any regulatory agency.

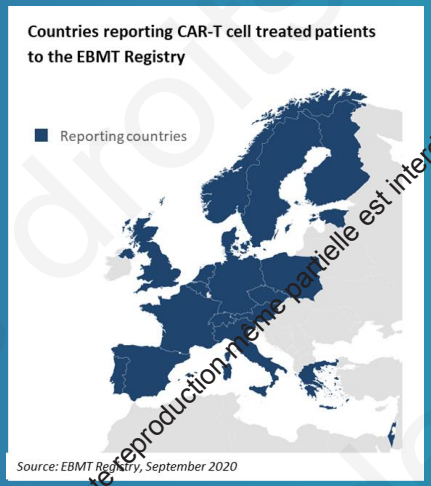
Registration of patients treated with CAR-T Cells in France





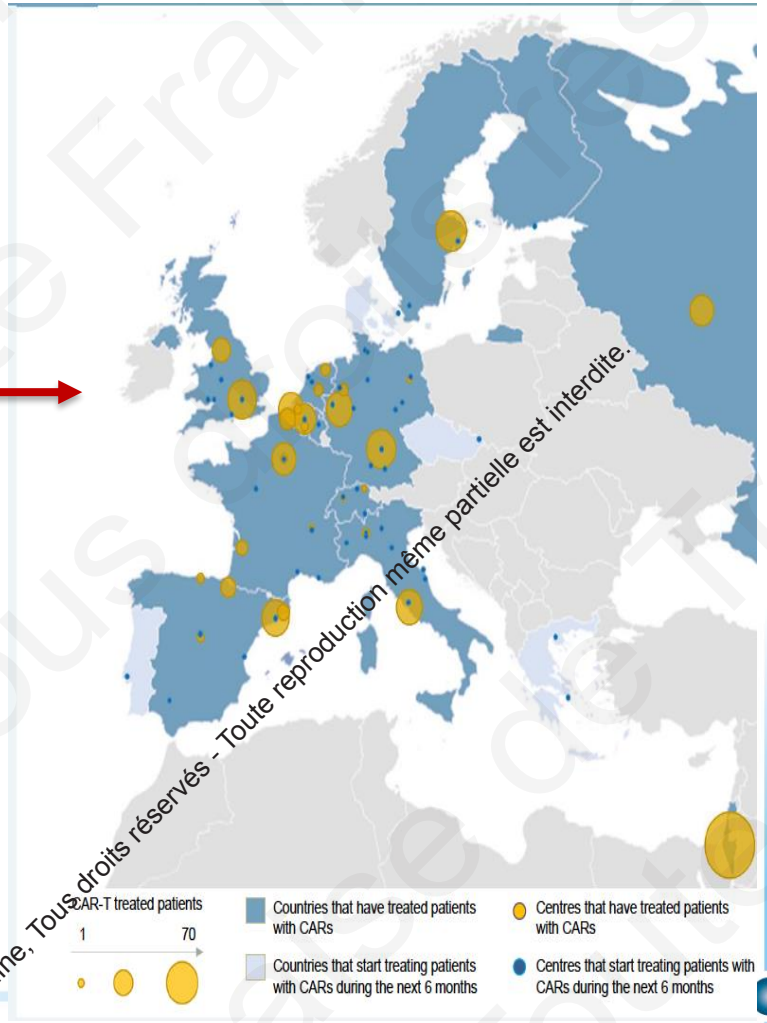
Registration of patients treated with CAR-T Cells in Europe





The European landscape for CAR-T Cells development

- Participation of EU centers and investigators to industry-sponsored clinical trials
- The EBMT Cellular Therapy & Immunobiology Working Party (CTIWP) survey 2018 (A Urbano-Ispizua et al; unpublished)
- The EBMT CTIWP Workshops
  - Newly designed CAR-T Cells and IECs
    - Targeting other tumor antigens, new CAR design …
  - “biosimilars”
    - Covering indications that are not covered by approved and commercially available CAR-T cells



Collection of blood mononuclear cells (1)

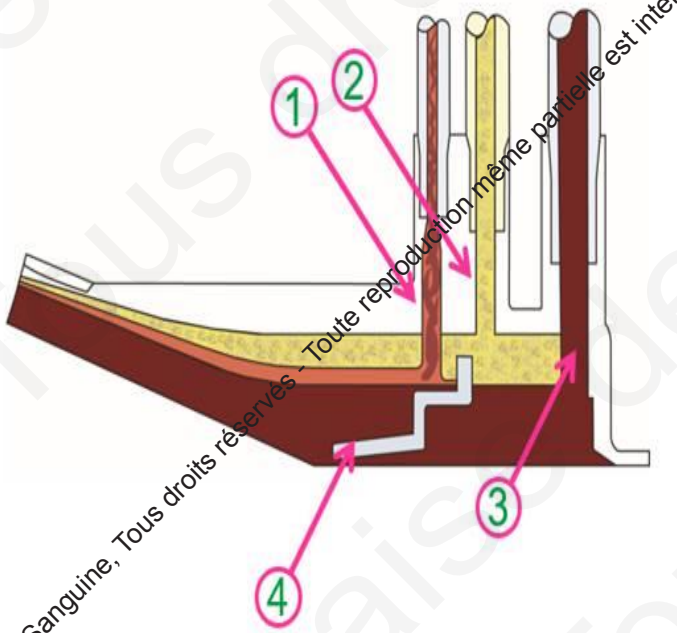
- Uses apheresis as a mean for extracorporeal separation of blood components and selective collection or depletion of one component
- Apheresis has many applications in medicines
- Many hematopoietic cellular therapies that are currently approved use peripheral blood mononuclear cells as the starting material for the manufacturing process

Cell Separation: Connector

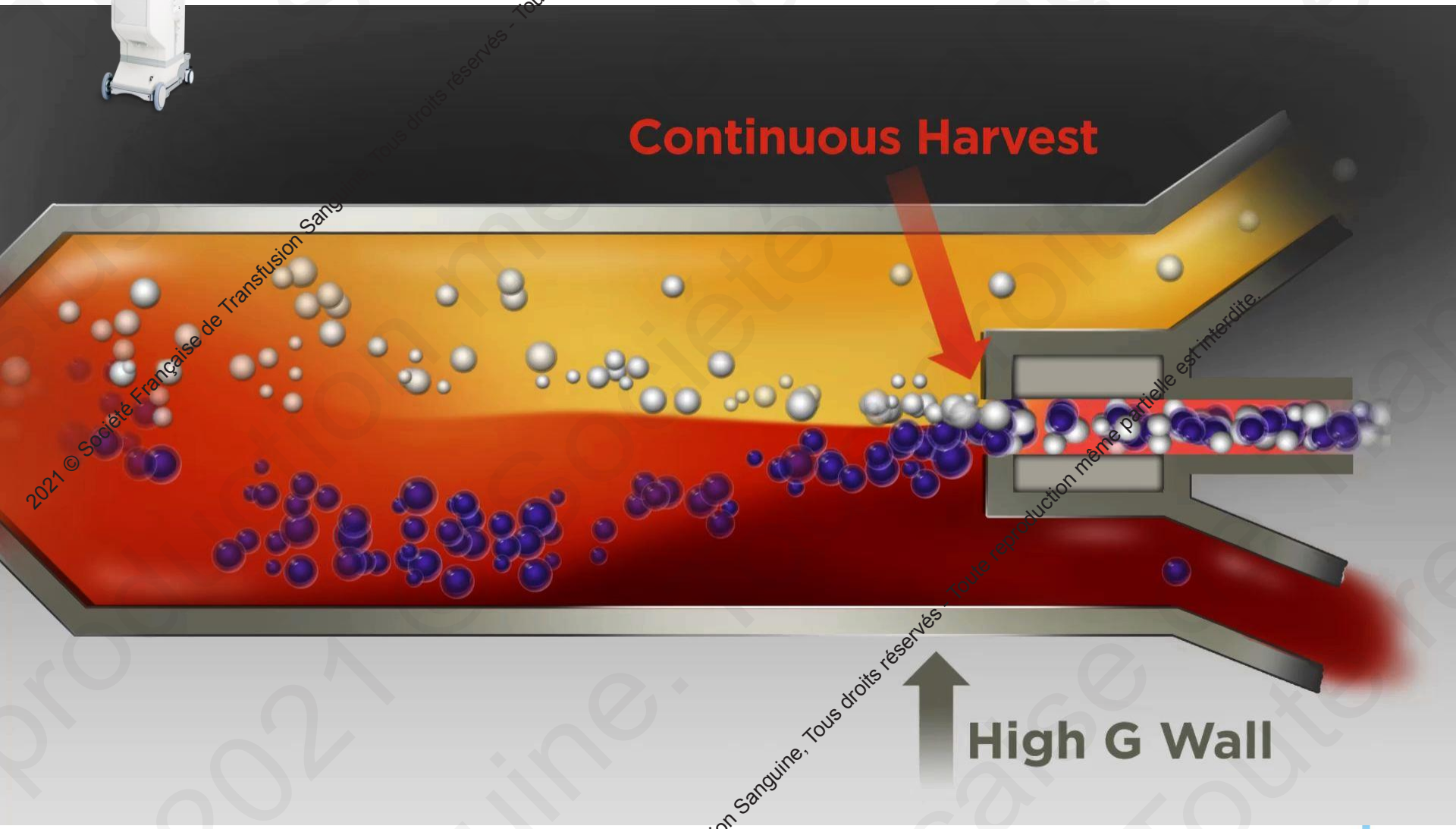
Centrifugal force separates the blood in the connector into layers based on the **specific gravity** of the cells:

- Platelets: 1.04 to 1.08
- MNC: 1.06 to 1.09
- RBC: 1.08 to 1.11

1. Collect port
2. Plasma port
3. RBC port
4. Skimmer dam



Continuous collection



Collection of blood mononuclear cells (2)

- **The disposables and programs used for the collection of autologous mononuclear cells with the intention to manufacture CAR-T Cells are similar to those used in the context of hematopoietic cell transplantation**

		Autologous	Allogeneic
Unmobilized	Source of immune effector cells	ATMPs off-line ECP	DLI
After mobilization treatment with rhG-CSF	Source of stem cells	auto-HCT ATMPs	allo-HCT

Factors that affect apheresis efficiency

- **Patient related factors**
  - Circulating blood counts, including absolute lymphocyte counts
  - Medical history, prior treatments
- **Procedural aspects**
  - Total blood volume to be processed
  - inlet flow rate, collect flow rate, AC ratio … can be adjusted by the operator
  - [Number of sessions]

High rates of success for collection

- **Published reports suggest close to 100% success rates in adults, even when severely lymphopenic**
- **Adverse events are usually mild and similar in nature to those encountered when blood mononuclear cells are collected for other purposes than manufacturing of CAR-T Cells**
- **Management of low-weight pediatric patients require specific measures**

Factors that affect T-cell functionality

- **Prior treatments**
- **Ongoing treatments**
  - Many treatments must be discontinued several days before apheresis is performed, including steroids, other immune-suppressive drugs, immune modulators …
- **Contaminants in the mononuclear cell fraction:**
  - Stroncek DF et al. Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptorT cells. *Cytotherapy*, 2016; 18: 893–901. http://dx.doi.org/10.1016/j.jcyt.2016.04.003

Type of therapy	EBMT/EHA recommendations	Comments
Allo-HCT	Patients should be off immunosuppression and GvHD-free	A minimum of one month is recommended with the requirement to be GvHD-free and off immunosuppression
DLI	At least 4 weeks	6-8 weeks may be safer to rule out any GvHD
High-dose chemotherapy	3-4 weeks	Recovery from cytopenias is required
Intrathecal therapy	One week	
Short-acting cytotoxic/anti-proliferative drugs	3 days	Recovery from cytopenias is required
Systemic corticosteroids	Minimum of 3 days but ideally 7 days	ALC $\geq 0.2 \times 10^9$/L is recommended

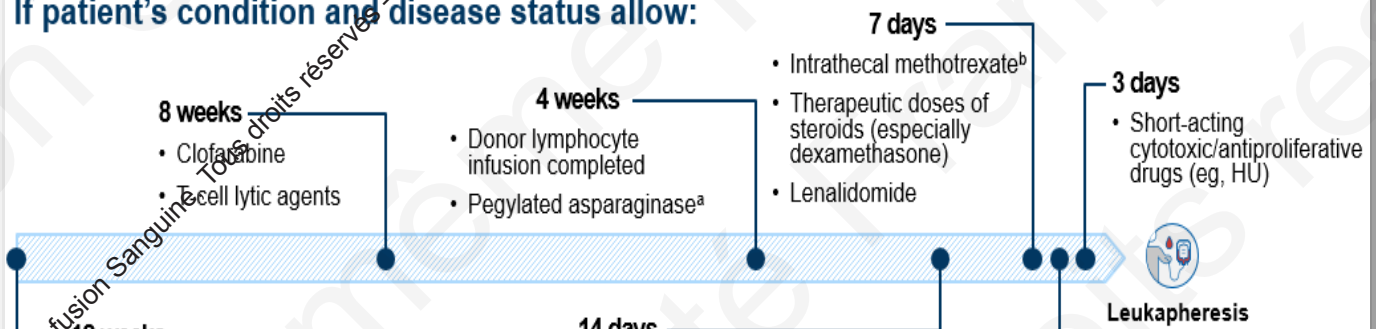
Table 4. Washout period before leukapheresis [adapted from Kansangra et al, BBMT 2019[15]] Key: *Allo-SCT: allogeneic stem cell transplantation; GvHD: graft versus host disease; DLI: donor lymphocyte infusion; ALC Absolute Lymphocyte Count.*

Novartis Oncology

Guidance for Therapy and Drug Washout Prior to Leukapheresis for Tisagenlecleucel Manufacture

Washout Prior to Leukapheresis: Recommendations[1-4]

If patient's condition and disease status allow:



Special considerations:

- Bendamustine and fludarabine: Washout ≥12 weeks[c]
- Alemtuzumab and ATG: Washout ≥6 months[d]

[a]In the CASSIOPEIA trial (NCT03876769) for pALL, the recommended washout period is 14 days.[3]
[b]If indicated, intrathecal cytarabine can be given up to a day prior to leukapheresis. For an intravenous cytarabine dose <100 mg/m^2, a washout of 7 days is recommended; for a dose ≥100 mg/m^2, a washout of 14 days is recommended.
[c]For bendamustine and fludarabine, allow adequate washout and avoid use for ≥12 weeks prior to leukapheresis due to the potential long-term effects on T cells; however, there are limited data in the context of CAR-T cell therapy for these agents. Please refer to the 12-week washout section for further information.
[d]Alemtuzumab and ATG (T-cell lytic agents): Allow adequate washout and avoid use for ≥6 months prior to leukapheresis and consider the potential prolonged effects of T cells. Please refer to the 6-month washout section for further information.
[e]Although blinatumomab half-life is short (~2 hours), it is recommended to washout 1 to 2 weeks prior to leukapheresis.
ATG, anti-thymocyte globulin; CAR, chimeric antigen receptor; GVHD, graft-vs-host disease; HU, hydroxyurea; pALL, pediatric acute lymphoblastic leukemia.

1. Yakoub-Agha I, et al. *Haematologica*. 2020;105(2):297-316; 2. Jain T, et al. *Biol Blood Marrow Transplant*. 2019;25(12):2305-2321; 3. Data on file. CCTL019G2201J Protocol v00. Novartis Pharmaceuticals Corp; February 11, 2020; 4. Data on file. Novartis Pharmaceuticals Corp. 2020.

NOVARTIS | Reimagining Medicine

CAR-T Cells attributes and clinical outcome ?

- Rossi J et al. Preinfusion polyfunctional anti-CD19 chimeric antigen receptor T cells are associated with clinical outcomes in NHL. *Blood*. 2018;132:804-14. DOI 10.1182/blood-2018-01-828343.
- Finney OC et al. CD19 CAR T cell product and disease attributes predict leukemia remission durability. J Clin Invest. 2019; 129:2123-32. https://doi.org/10.1172/JCI125423

Shipment of the collected cell product

- **From the cell processing facility teaming up with the collection facility**
- **In compliance with the manufacturer's instructions**
- **The collected cell product is handled to a courier mandated by the manufacturer**
- **Depending on manufacturer's instructions, the cells may be shipped "fresh" or following on-site cryopreservation**
  - Cryopreservation allows for more flexibility
  - Cryopreservation raises the issue of precautionary collection of blood MNC

Change of status during the manufacturing process

- The apheresis product qualifies as human cells
- CAR-T Cells qualify as a Medicinal Product

NOVARTIS

Manufacturing Site :
Novartis Pharma Stein AG
Schaffhauserstrasse
CH-4332 Stein
Switzerland
Manufacturing licence no.: 511177-102616884
GMP certificate no.: GMP-CH-1000750

CERTIFICATE OF ANALYSIS

Batch Number: STAFRS1

Product Name:	CTL019 tisagenlecleucel autologous T-cell suspension in Infusion bag
Indication:	DLBCL

Cell Collection & Cell Processing Facilities act as suppliers of the medicinal product manufacturer

- **Audits & re-audits of Cell Collection & Cell Processing facilities**
  - Document control (SOPs)
  - Tour of facilities
  - Training / habilitation of personnel …

- **Same approach than for FACT-JACIE Accreditation**

Published Ahead of Print on November 21, 2019, as doi:10.3324/haematol.2019.229781.

haematologica
Journal of The Ferrata Storti Foundation

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)

by Ibrahim Yakoub-Agha, Christian Chabannon, Peter Bader, Grzegorz W. Basak, Halvard Bonig, Fabio Ciceri, Selim Corbacioglu, Rafael F. Duarte, Hermann Einsele, Michael Hudecek, Marie José Kersten, Ulrike Köhl, Jürgen Kuball, Stephan Mielke, Mohamad Mohty, John Murray, Arnon Nagler, Stephen Robinson, Riccardo Saccardi, Fermin Sanchez-Guijo, John A. Snowden, Micha Srour, Jan Styczynski, Alvaro Urbano-Ispizua, Patrick J. Hayden, and Nicolaus Kröger

Haematologica 2019 [Epub ahead of print]

Citation: Ibrahim Yakoub-Agha, Christian Chabannon, Peter Bader, Grzegorz W. Basak, Halvard Bonig, Fabio Ciceri, Selim Corbacioglu, Rafael F. Duarte, Hermann Einsele, Michael Hudecek, Marie José Kersten, Ulrike Köhl, Jürgen Kuball, Stephan Mielke, Mohamad Mohty, John Murray, Arnon Nagler, Stephen Robinson, Riccardo Saccardi, Fermin Sanchez-Guijo, John A. Snowden, Micha Srour, Jan Styczynski, Alvaro Urbano-Ispizua, Patrick J. Hayden, and Nicolaus Kröger. 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). Haematologica. 2019; 104:xxx doi:10.3324/haematol.2019.229781

GUIDELINE ARTICLE

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]

Ferrata Storti Foundation

Haematologica 2018
Volume 105(2):297-316

Table 11. Monitoring of patients during medium-term follow-up.

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 3 months, three monthly thereafter in first year	Guide to anti-infective prophylaxis
CAR T-cell monitoring where kits are available for routine monitoring of anti-CD19 CAR T cells	CAR T-cell persistence	Peripheral blood flow cytometry or transgene by molecular methods as clinically indicated	Not recommended by CAR T-cell manufacturers

FBC: full blood count; LDH: lactate dehydrogenase; CRP: C-reactive protein; CMV: cytomegalovirus; EBV: Epstein-Barr virus; IV: intravenous; CAR: chimeric antigen receptor.

TITLE:

‘Management of Adults and Children receiving CAR T-cell therapy: 2021 Best Practice Recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA)’

AUTHORS AND AFFILIATIONS:

Patrick J. Hayden[1]*, Claire Roddie[2, 3]*, Peter Bader[4], Grzegorz W. Basak[5], Halvard Bonig[6], Chiara Bonini[7], Christian Chabannon[8], Fabio Ciceri[9], Selim Corbacioglu[10], Rose Ellard[11], Fermin Sanchez-Guijo[12], Ulrich Jäger[13], Martin Hildebrandt[14], Michael Hudecek[15], Marie José Kersten[16], Ulrike Köhl[17], Jürgen Kuball[18], Stefan Mielke[19], Mohamad Mohty[20], John Murray[21], Tamar Nagler[22], Jeremy Rees[3, 23], Catherine Rioufol[24], Riccardo Saccardi[25], John Snowden[26], Jan Styczynski[27], Marion Subklewe[28], Sophie Terwel[29], Catherine Thieblemont[30], Max Topp[15], Álvaro Urbano Ispizua[31], Daniel Chen[3], Radovan Vrhovac[32], John G. Gribben[33], Nicolaus Kröger[34], Hermann Einsele[15] and Ibrahim Yakoub-Agha[35]

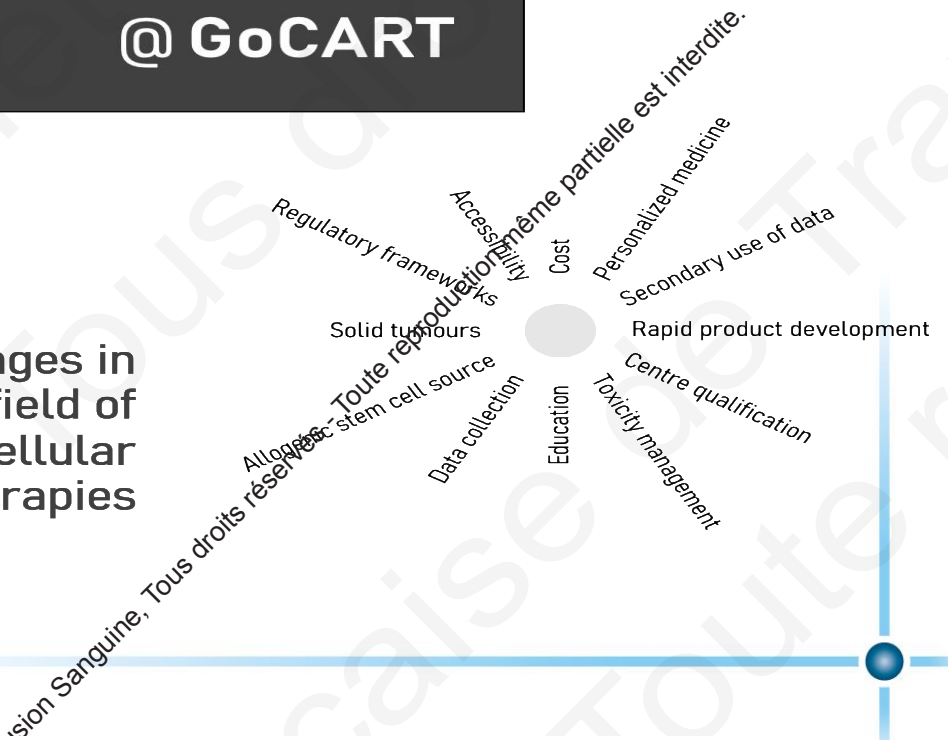
* Participated equally to this work and should be considered as first author.

We don't aim to break silos down, but to connect them @ GoCART

GoCART COALITION

Contact: sofie.terwel@ebmt.org ;
y.cabrerizo@ehaweb.org

Challenges in the field of cellular therapies



Conclusions (1)

- **Apheresis is a key step in the manufacturing process**
- **There is currently no specific protocol for mononuclear cell collection in view of manufacturing autologous CAR-T Cells**
  - Proper execution of apheresis relies on the implementation of a strict Quality Management System (QMS)
- **Collection of the required number of MNC / CD3+ cells is feasible in the vast majority of cases, even in severely lymphopenic patients**

Conclusions (2)

- **Beyond numbers, functionality of collected cells likely affects:**
  - The success of the manufacturing process
  - The functionality of manufactured CAR-T Cells
  - The clinical activity of the infused medicinal product, including in vivo expansion and persistence
- **Importance to discontinue all treatments that affect T cell functions before proceeding to apheresis**
- **Biological monitoring of patients and quality controls on collected cell products will provide important information**

Acknowledgements

- Institut Paoli-Calmettes Comprehensive Cancer Center
  - Collection and processing facility
    - M Barbaroux, MP d'Ingrando, A Festa, J Georgeton, S Paquis, M, F Braham, K Sestier
    - A Granata, T Pagliardini, V Maisano
    - S Thévenet, B Mfarrej, B Calmels, C Lemarié, E Kerjean, F Darbon, F Benizri, E Fougereau …
  - Clinical facility
    - S Fürst, F Legrand, D Blaise, S Harbi, A Granata, R Devillier, A Collignon, C Montes de Occa, JM Schiano de Collela, R Noel, G Brisou, T Aurran-Schleinitz, AM Stoppa ….
- EBMT
  - ExCom & Board of Association
  - Registry Office (London, UK)
  - Clinical Trials Office (Leiden, NL)
  - Administrative office (BCN, Spain)
  - GoCART consortium
- National Transplant professional associations
  - SFGM-TC

Thank you

chabannonc@ipc.unicancer.fr; christian.chabannon@univamu.fr

sion Sanguine, Tous droits réservés - Toute reproduction même partielle est interdite.