Aspetti trasfusionali e standardizzazione della raccolta dei linfociti finalizzati alla produzione di CAR-T

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Regulatory background

Evolution toward ATMPs

Context:

- **New regulatory constraints:**
  the "Advanced Therapy Medicinal Products" (ATMPs) (Directive 2001/83/CE)

  ➔New class of drugs, but still **DRUGS!**
- **Defining an optimal organizational approach** in order to support this evolution:
  - ✓ Patients deserve to receive such innovative treatments
  - ✓ But these new drugs are not standard ones, cover multiple regulations, and thus need to fit with several requirements
- A need expressed by institutional and industrial staff for developing structures able to **manage CAR-T as soon as the early clinical stages**

ATMP, Advanced Therapy Medicinal Products; CAR-T, chimeric antigen receptor therapy.
European Medicines Agency. Directive 2001/83/EC. Available at:
https://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:02001L008320121116&from=EN.
Accessed October 2019

Advanced Therapy Medicinal Products (ATMPs)

- **Biological medicinal products**
  (Annex I, Directive 2001/83/EC)

- **Four categories**
  - Gene Therapy Medicinal Product (GTMP)
  - Somatic Cell Based Medicinal Product (sCBMP)
  - Tissue-Engineering Product (TEP)
  - Combined ATMP

CAR-T cells

ATMP, Advanced Therapy Medicinal Product; CAR-T, chimeric antigen receptor therapy.
European Medicines Agency. Directive 2001/83/EC.
Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02001L0083-20121116&from=EN. Accessed October 2019.

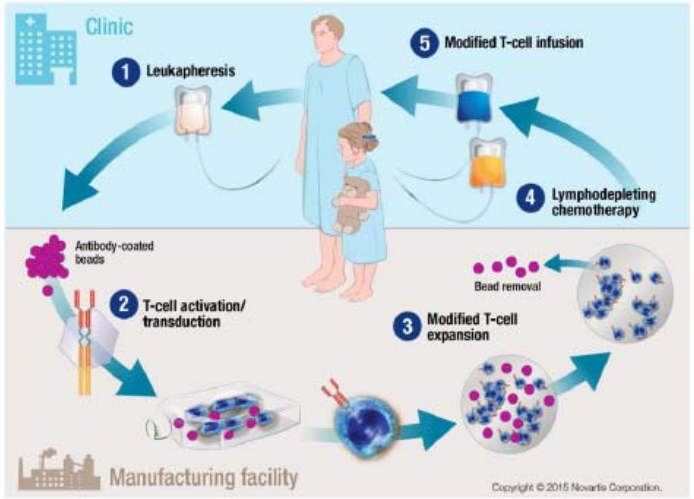
CAR-T cells hospital management

Apheresis product

Apheresis department

Cell product

Cell Therapy Unit



Drug

Pharmacy

Raw material = living cells

Company = GMP manufacturing plant

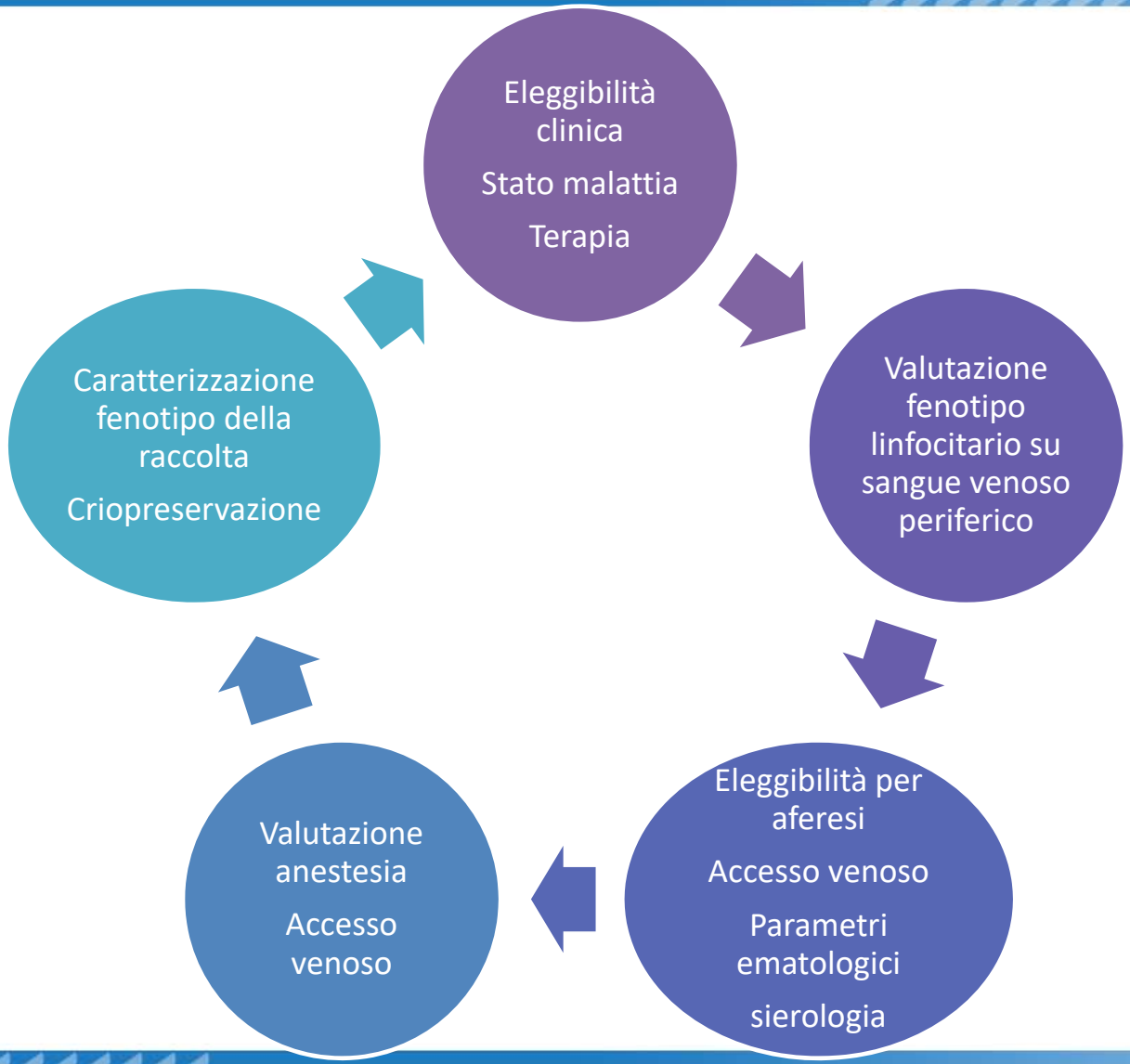
Final product = modified raw material = GMO cell suspension

Company = GMP manufacturing plan

A drug, after multiple different status

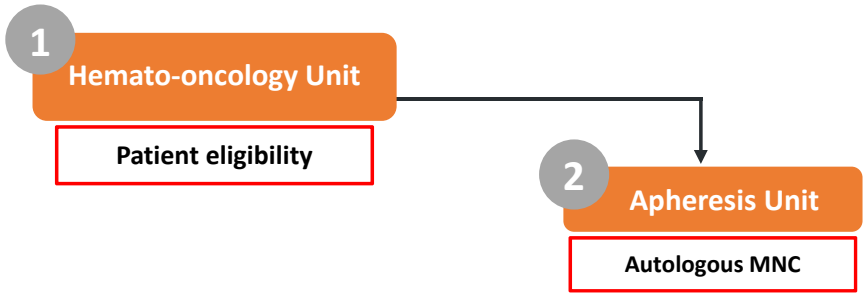
CAR-T, chimeric antigen receptor therapy; GMO, genetically modified; GMP, good manufacturing practices.
Porter DL, et al. *N Engl J Med* 2011;365:725–733; Porter DL, et al. *J Cancer* 2011;2:331–332;
Kalos M, et al. *Sci Transl Med* 2011;3:95ra73.

Percorso multidisciplinare



Il circuito delle CAR-T cell autologhe

Hospital authorized for HSC graft & GMO use



GMO, genetically modified; HSC, hematopoietic stem cell; MNC, mononuclear cell.
Jérôme Larghero Personal Communication.

Qualification of the apheresis product

1. Before the apheresis

- Donor consent
- Certificate of donor aptitude for apheresis
- Donor serology <30 days

Autologous graft
- Hepatitis B virus
- Hepatitis C virus
- HIV 1 & 2 viruses
- HTLV 1 et 2
- Syphilis

- ABO blood group & Rhesus

HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus.
Jérôme Larghero Personal Communication.

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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)

Checklist before the apheresis

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™) to seven (Yescarta™) 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 *et al* (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)		$>1.0x10^9$/L	Consistent with recovery from prior chemotherapy
Absolute Lymphocyte count (ALC)		$> 0.2x10^9$/L*	Higher count required in small children. Of note, $0.2x10^9$/L $CD3^+$ count is the minimum threshold
Platelet count		$> 30x10^9$/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

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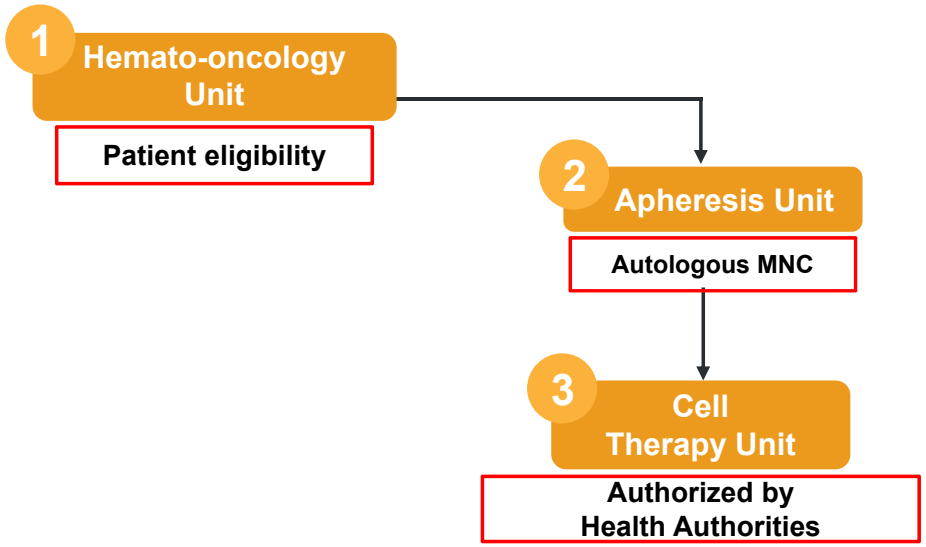
Hospital authorized for HSC graft & GMO use



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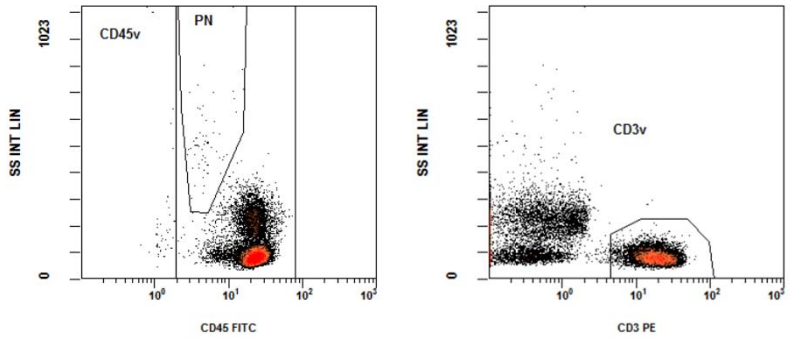
Jérôme Larghero Personal Communication.

Qualification of the apheresis product by the Cell Therapy Unit

1. After the apheresis

- Qualification of MNC by the Quality Control department of the CTU:
  - Cell count
  - Immunophenotype (flow cytometry): CD45+/CD3+
  - Cell viability
  - Sterility



CD, cluster of differentiation; CTU, Cell Therapy Unit; MNC, mononuclear cell.
Jérôme Larghero Personal Communication.

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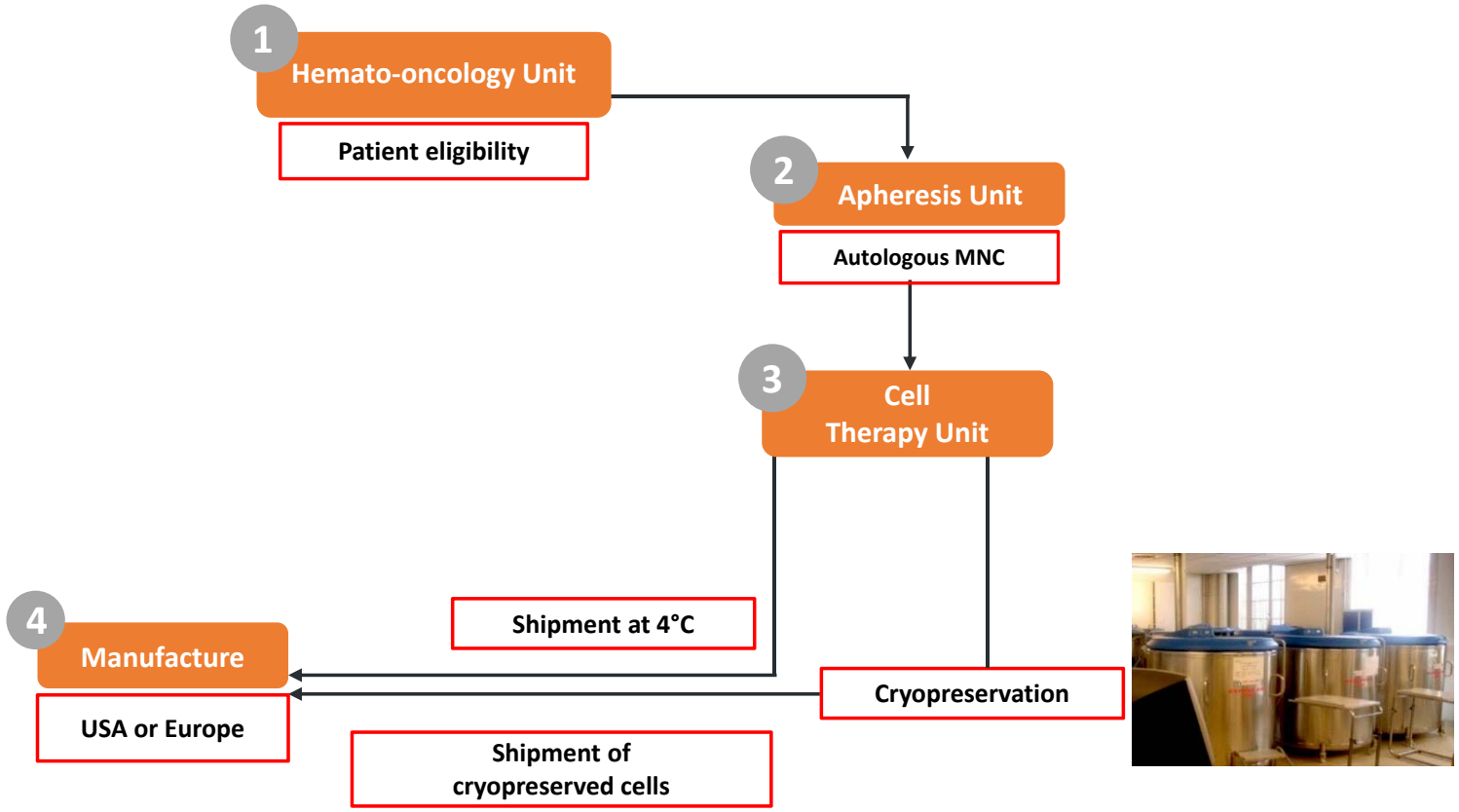

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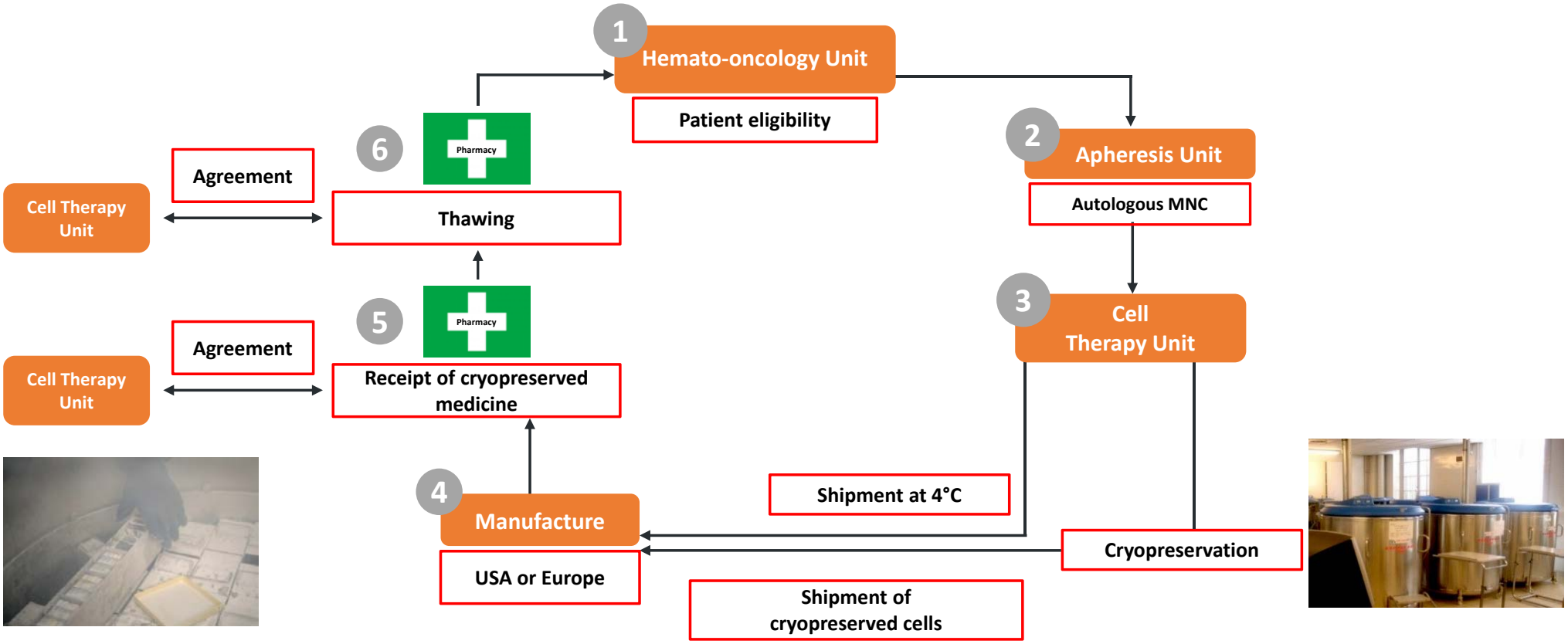

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Minimum required tests

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

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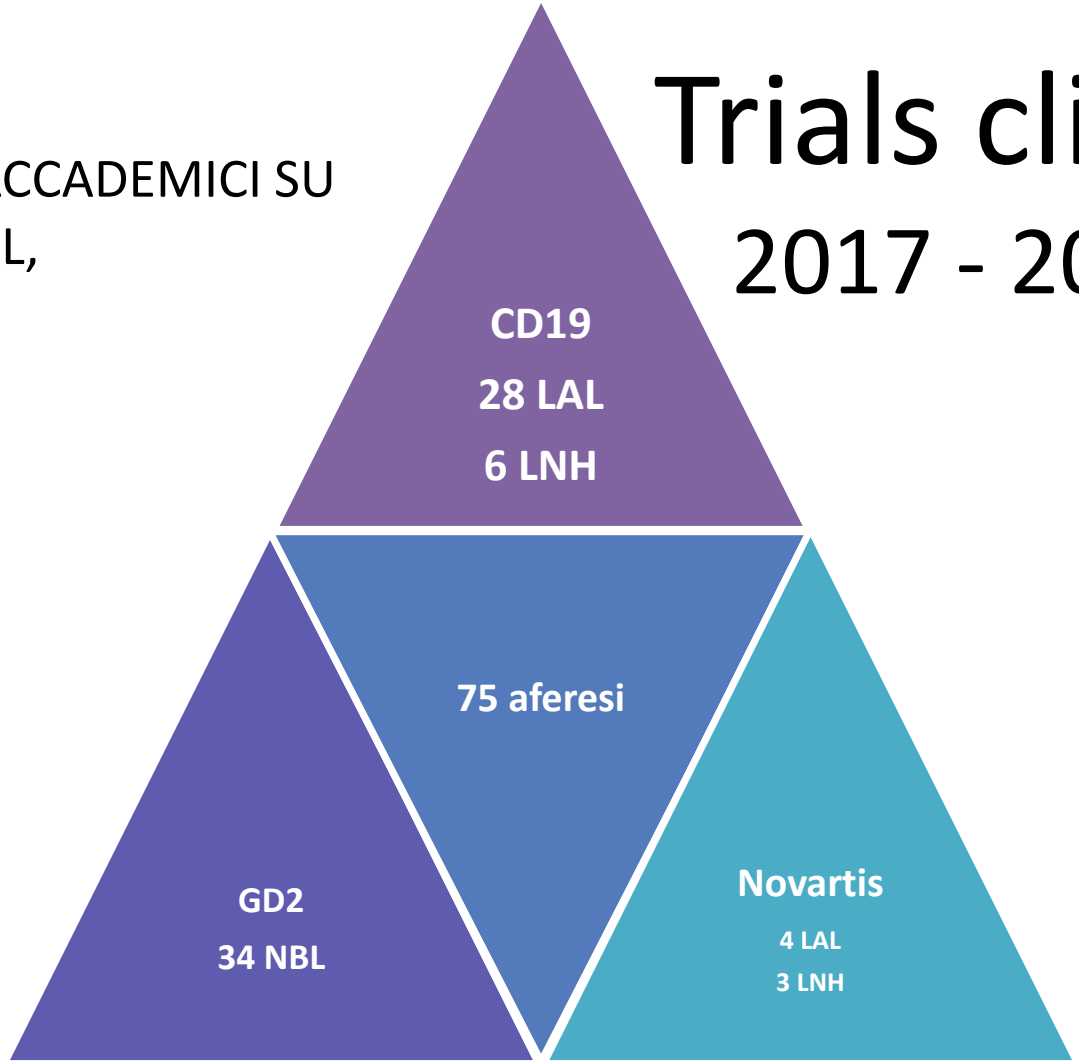
OPBG
Dal 2018, 2 TRIALS ACCADEMICI SU CAR T per ALL B e NBL,
2 TRIALS PHARMA

Trials clinici 2017 - 2019



Wash out period before leukapheresis

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 *et al* (12) Regardless of timing, an ALC>0.2 x10^9/L is preferable given the likely effect of recent corticosteroids on lymphocyte quality

Yakoub-Agha, Haematologica 2019

OPBG – Apheresis for CAR T manufacturing

	totali	ALL	NHL	NBL
Pazienti	75	32	9	34
N° raccolte	75	32	9	34
Età anni				
Media	10.4	11.2	13	8.7
Range	3-25	3-25	8-18	3-23
Peso Kg				
Media	36.7	38.6	60.4	26.2
DS	23,9	23.7	21.2	16.8
range	11-106	13.6-82	22.6-106	11-73

Collection Efficiency (CE)

- CE is used to estimate the volume to be processed to achieve the target dose of T-cells
- For those manufacturers indicating target doses for mononuclear cells, CE can be calculated accordingly
- Not all commercial CAR T-cell manufacturers provide target cell counts for the apheresis product; some request the processing of a certain Blood Volume, regardless of patient size and lymphocyte counts
- 1- x10e9 T-cells is usually sufficient to start CAR T-cell manufacturing

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

Processing

Equation 1. Calculating Estimated Minimum Total Blood Volume to be Processed[a]

$$\text{Estimated minimum blood volume to be processed (L)} = \frac{1\times10^{9}\ \text{(cells)}^{b}}{\text{Collection efficiency}^{c} \times \text{Peripheral CD3}^{+}\ \text{lymphocyte count (cells/µL)} \times 10^{6}\ \mu\text{L/L}}$$

Peso (kg)	0-15	15-30	30-106
Processato (ml) Media range	 2900 1800 – 4550	 4346 2120- 6771	 6377 2300-11300
Volemie Media range	 2.7 1.6 – 4.3	 2.7 1.3 – 4.3	 1.5 0.5 – 3.3

Linfo 10°3/µl pre	< 500	500-1000	>1000
Processato (ml) Media DS range	 6.600 2000 2200 - 11.300	 5.800 2090 1900 - 9900	 4000 1100 1800 - 6500

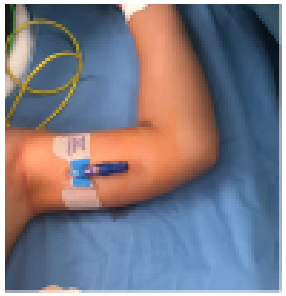
Venous access

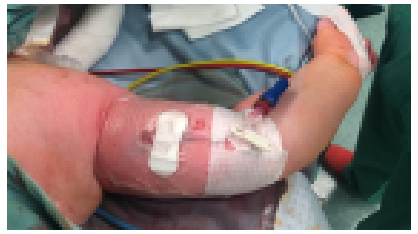
- CVC bilume
  - 39 paz
- Accesso periferico
  - 19 paz di cui 2PGm
- CVC + VP
  - 17 paz

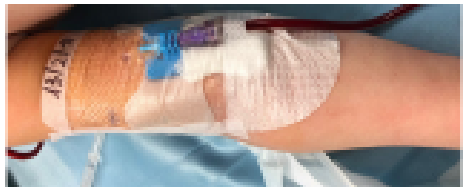
BREVE TERMINE (< 1 mm)

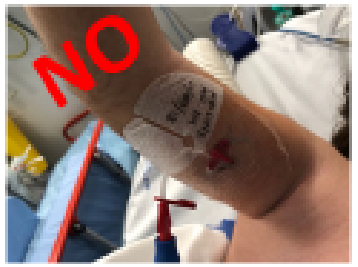
Agocannule periferiche lunghe a permanenza (Mini-midline)

- Monolume
- Posizionamento per puntura diretta v. avambraccio
- Posizionamento ecoguidato v. profonde del braccio
- Tecnica Seldinger diretto (Leader flex/cath)
- Tecnica Seldinger coassiale (Power-Glide)
- Punta tratto brachiale v. ascellare
- Anche power injectable (Power-Glide)
- Solo farmaci **NON** flebitogeni
- Uso intra-ospedaliero









PowerGlide (BARD)

Leader flex (Vygon)

Leader cath (Vygon)

Anticoagulant

- Anti-coagulation is initially achieved with ACD-A at a 1:10-1:12 ratio
- Additional use of heparin should be considered case by case
- The amount of ACD-A allowed per minute and hence, inlet flow, is limited by the patient's total blood volume
- Electrolyte shifts should be monitored regularly and, if necessary, corrected with i.v. or oral electrolytes (mostly calcium and potassium)

PROCEDURE AFERETICHE SECONDO STANDARD PER PRODOTTO NOVARTIS

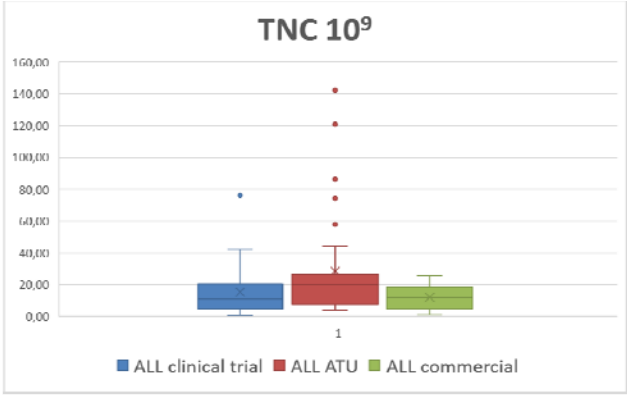
	Specification Values
$CD3^+$ lymphocyte count	≥ 1 x 10^9 $CD3^+$ cells
TNC count	≥ 2 x 10^9 TNC
CD3+% of TNC	≥ 3%

CD3 + lymphocyte count SD Range	**1.6 X 10°9 CD3+ cells** 0.5 x 10°9 CD3+ cells 0.8 – 2.3 x 10°9 CD3+ cells
TNC count x 10°9 SD range	**4 x 10°9 CD3+ cells** 2 x 10°9 CD3+ cells 2 -7 x 10°9 CD3+ cells
CD3+% of TNC SD range	**41 % CD3+** 5% CD3+ 35 – 47 % CD3+

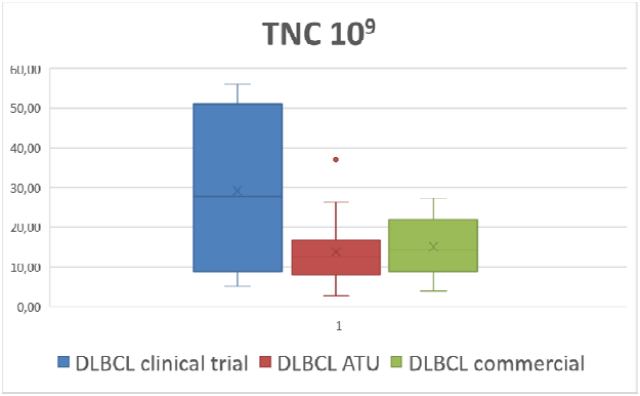
Quality controls of apheresis products

Total nucleated cells

ALL
n=67 patients



DLBCL
n=48 patients

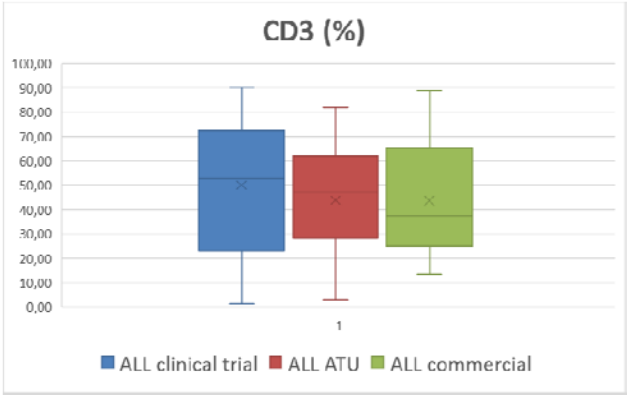


ALL, acute lymphoblastic leukemia; ATU, Autorisation Temporaire d'Utilisation; DLBCL, diffuse large B-cell lymphoma; TNC, total nucleated cell.
Jérôme Larghero Personal Communication.

Quality controls of apheresis products

CD3⁺ (%)

ALL
n=67 patients



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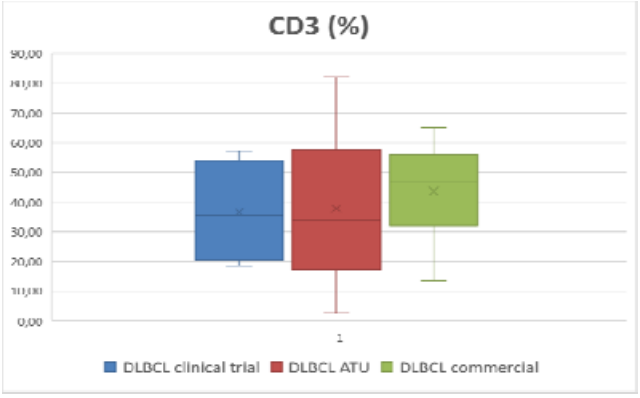

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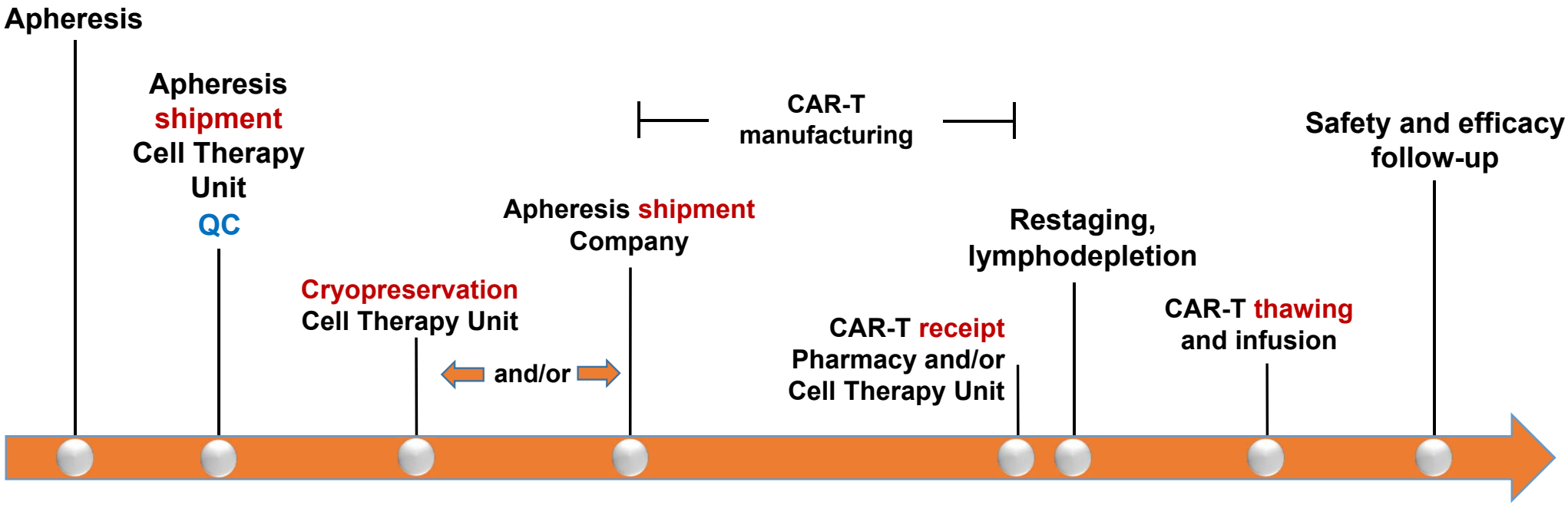
Several steps “at risk”!



CAR-T, chimeric antigen receptor therapy; QC, quality control.

A complex process

Several reasons that might affect the quality of the cell product:

Before and during apheresis:

- Previous treatments
- Disease status
- Patient age
- Cell number, populations/phenotypes
- Apheresis duration
- Microbiological controls
- Labelling

During the manipulation of the apheresis product:

- Time to cryopreservation
- Cryopreservation
- Storage
- Manipulation of the cryopreserved product at the time of shipment
- Shipment of the apheresis product either fresh or cryopreserved
- Labelling

A validated procedure to ensure identification and tracking of the product at any step must be in place

A complex process

Management of the apheresis product

Cryopreservation challenge for the Cell Therapy Unit

- Has to be performed less than 24 h after apheresis. Easy to say, but….
- Cryopreservation process to be validated for different volumes of final cell product (depends on final cell concentration)
- Less than 30 mn of contact with cryoprotectant solution
- What about the regulation agencies authorization?

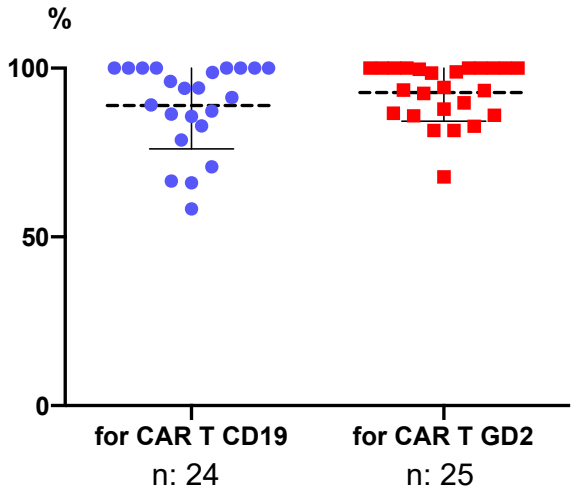
Storage challenges for Cell Therapy Units

- To be able to have dedicated racks in a tank in the vapour phase
- The bag must be clearly separated from other patients' products
- To train all staff involved in this activity

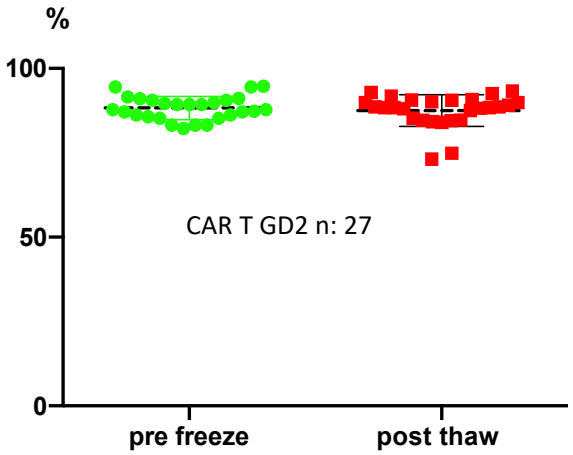
ATMP, Advanced Therapy Medicinal Product.

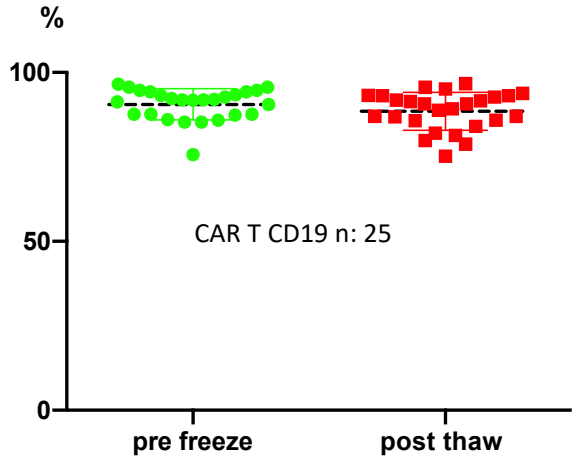
OPBG experience on cryopreservation of apheretic products and CAR T cells

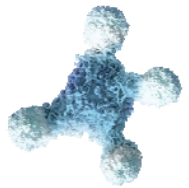
Post thaw recovery of viable MNC >90%



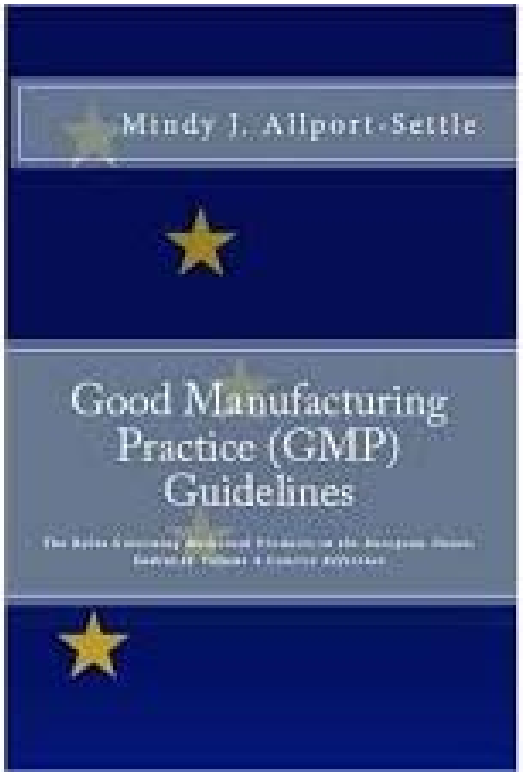
No difference in viability of CAR T cells before freezing and after thawing.







Out of specification (OOS)



- "**Exceptionally**, the administration of the cells/tissues that are contained in a cell/tissue based ATMP that is out of specification **may be necessary for the patient**.

- Where the administration of the product is **necessary to avoid an immediate significant hazard to the patient** and taking into account the alternative options for the patient and the consequences of not receiving the cells/tissues contained in the product, the supply of the product to the treating physician is justified.

- When the **request of the treating physician** is received, the manufacturer should provide the treating physician with its evaluation of the risks…"

Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products. November 2017. Available at: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf.

Conclusions

- Cell therapy product/ATMP regulation to deal with, but it is not the only issue!
- How to deal with CD3+ cell number at the time of harvest?
  - Other cell populations?
  - Other T-cell subsets?
- The cryopreservation process AND the cell thawing
  - Critical steps
  - What is really infused into the patient?
- A need to share with a multidisciplinary team

ATMP, Advanced Therapy Medicinal Product; CD, cluster of differentiation.

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