POINT-OF-CARE / DECENTRALIZED CAR-T CELL PRODUCTION

Yves BEGUIN

Dpt of Hematology, CHU & U of Liège, Belgium

CAR-T cells

Belgian legislation

Human Bodily Material
Collected by MD in hospitals

Loi du 19 décembre 2008 relative à l'obtention et à l'utilisation de matériel corporel humain destiné à des applications médicales humaines ou à des fins de recherche scientifique

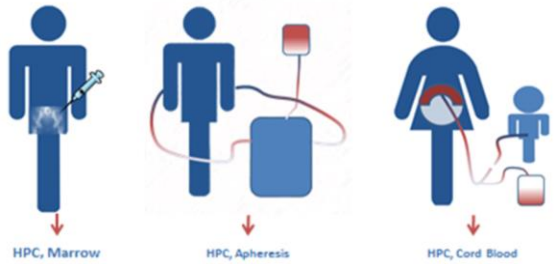
Scientific research

- Biobank
- No therapeutic use in humans

Therapeutic use

Non-substantial manipulation

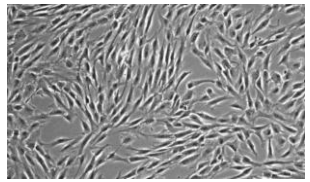
- Cell & tissue bank (hospital)
- Intermediate structure (commercial)

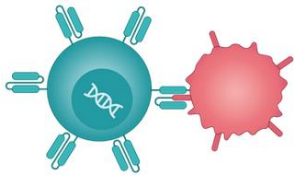


Substantial transformation

→ ATMP : Advanced Therapeutic Medicinal Product
(cells, genes, tissues, combinations)

→ GMP : Good Manufacturing Practice production





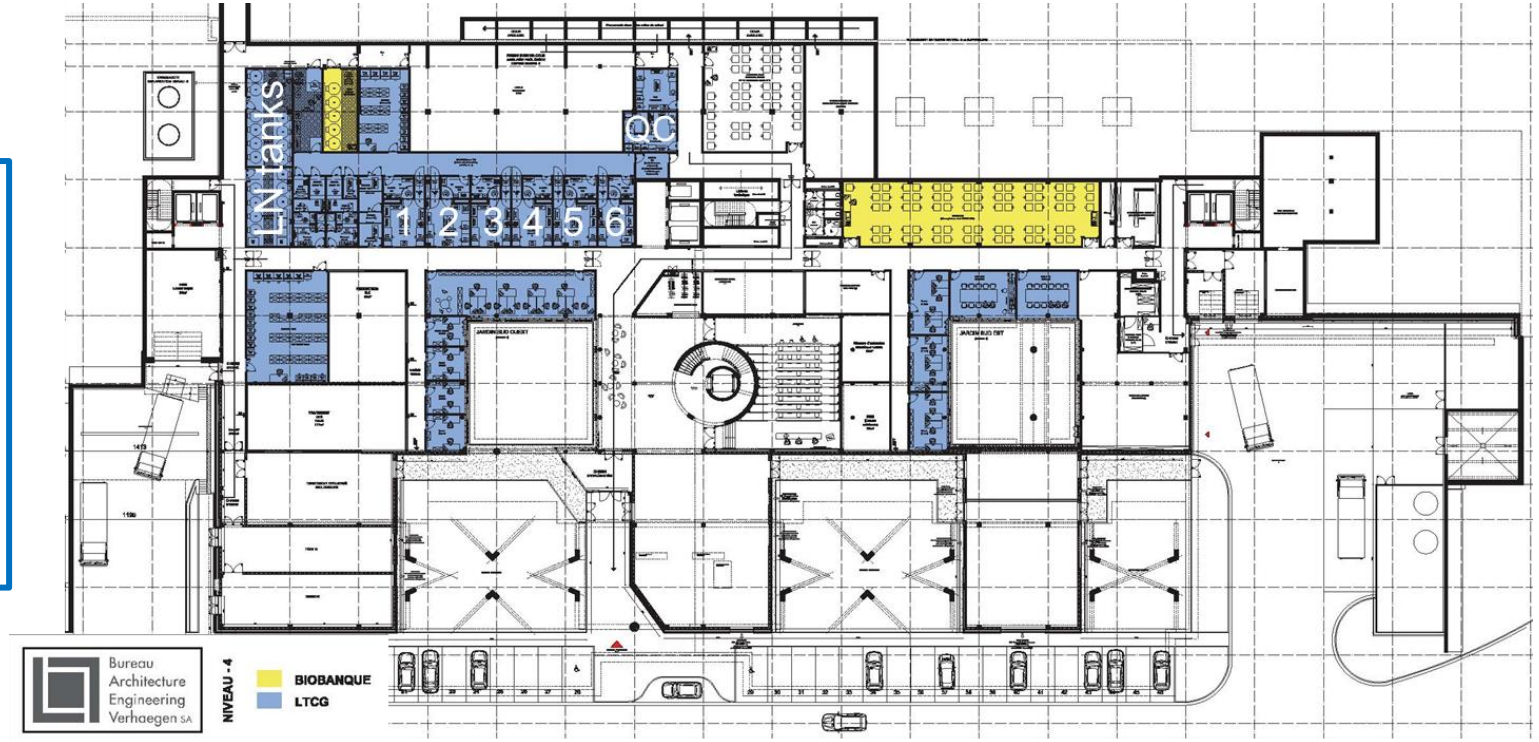
Liège Laboratory of Cell & Gene Therapy

Production & QC facilities

ICAB
Institut de Cancérologie Arsène Burny

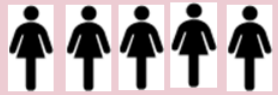
LTCG

6 GMP facilities
1 QC lab
75 m² of LN storage
Storage areas
Offices



Liège Laboratory of Cell & Gene Therapy

MSC : from clinical grade to GMP

Personnel +  • QC ≠ Production • Instrumentation • Personne qualifiée	**Locaux** Classe C ⬇ Classe B	**Habillage** 	**Monitoring environnemental** ↑↑↑↑ Fréquence, Nombre et type de prélèvements
Documentation + • Déviations • OOS • CCR GMP = Generate more paper	**Média simulation** **+ Média simulation** • 3X nouveau process • 2X/an par process • 1X/an par opérateur	**Validation** Validation du process ⬇ Validation process, contrôles de qualité, nettoyage Protocoles & rapports	**Réactifs & Consommables** + Quarantaine/libération (Spécifications) Evaluation fournisseurs
Equipements Maintenance annuelle ⬇ Procédures et IQ/OQ/PQ	**Echantillons rétention et référence** + d'échantillons (Identification et analyses)	**Contrôles de qualité** Changement de sous-traitants (certifiés GMP)	**Process** •Retrait antibiotiques •Trypsine recombinante (porcine) •Cellstacks

Lechanteur, Cells 2021

CAR-T cells

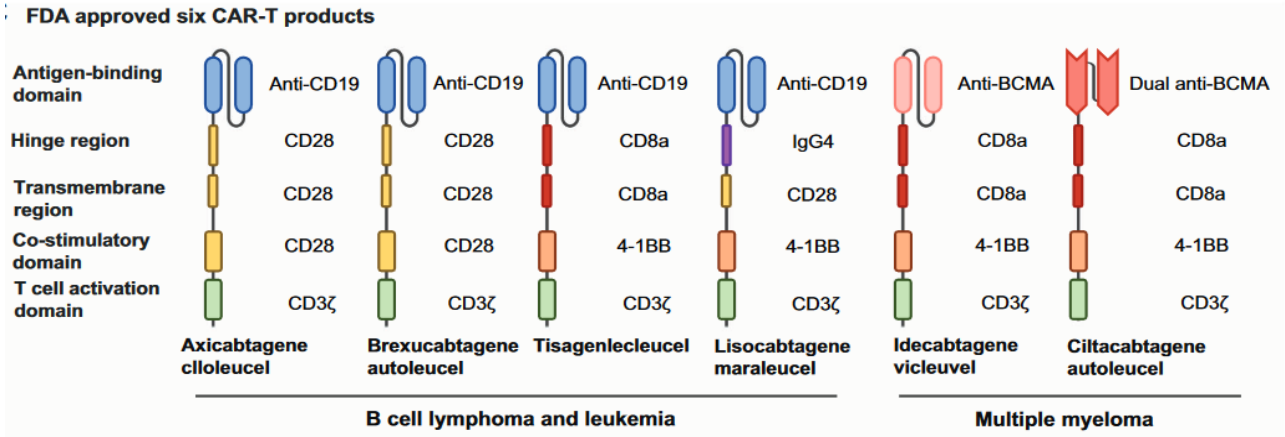
Classical commercial CAR-T cell production

Rossig, JCO 2023

CAR-T cells

B-cell malignancies : CAR-T cell products

Second generation CARs



Yescarta axicabtagene ciloleucel	Tecartus brexucabtagene autoleucel	Kymriah tisagenlecleucel	Breyanzi lisocabtagene maraleucel	Abecma idecabtagene vicleucel	Carvykti ciltacabtagene autoleucel

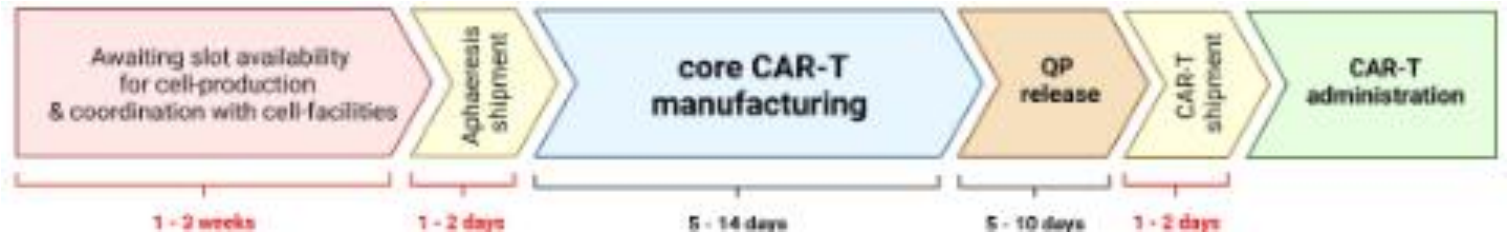
Co-stimulation

CD28

- faster in vivo expansion
- higher peak levels

4-1BB

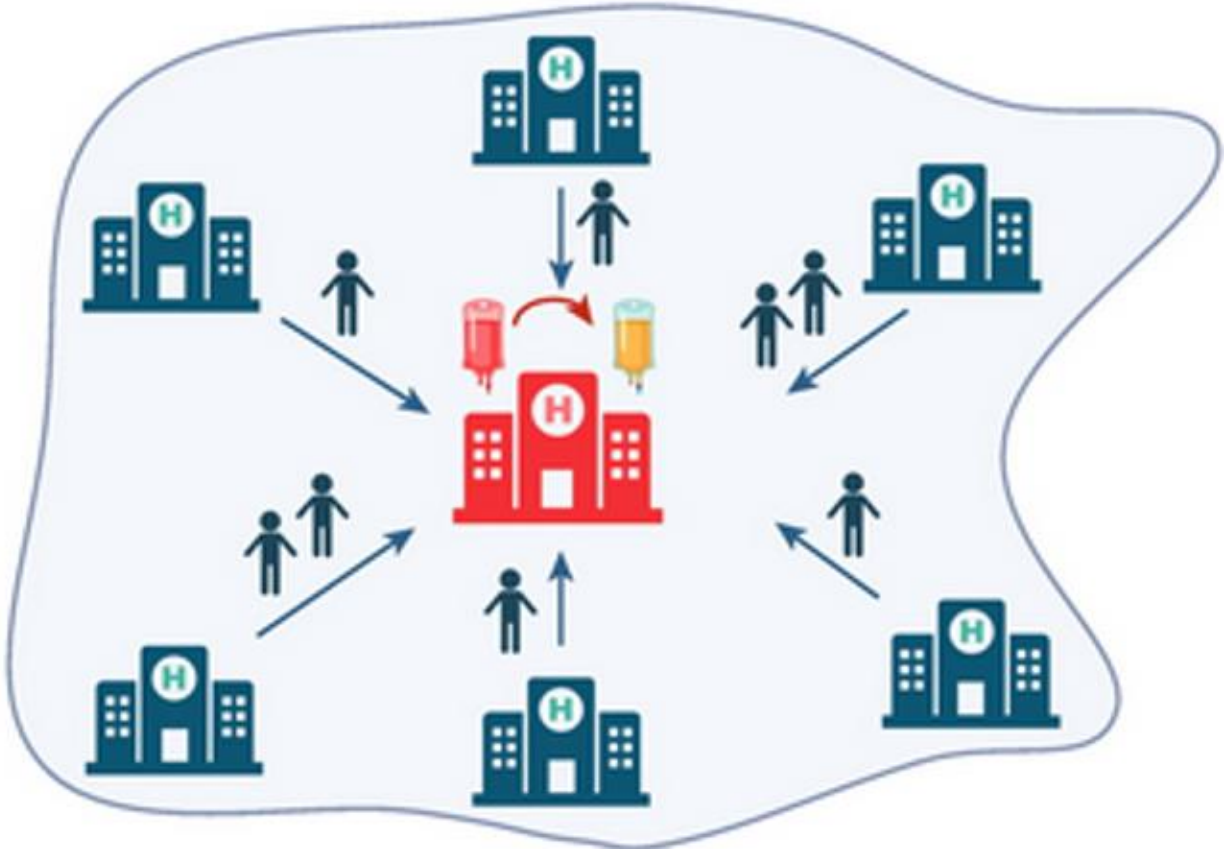
- drive towards CM phenotype
- longer persistence
- less exhaustion



Peng, Cell Mol Immunol 2024
Singh, Immunity 2023
Martinez-Cibrian, Front Immunol 2022

CAR-T cells

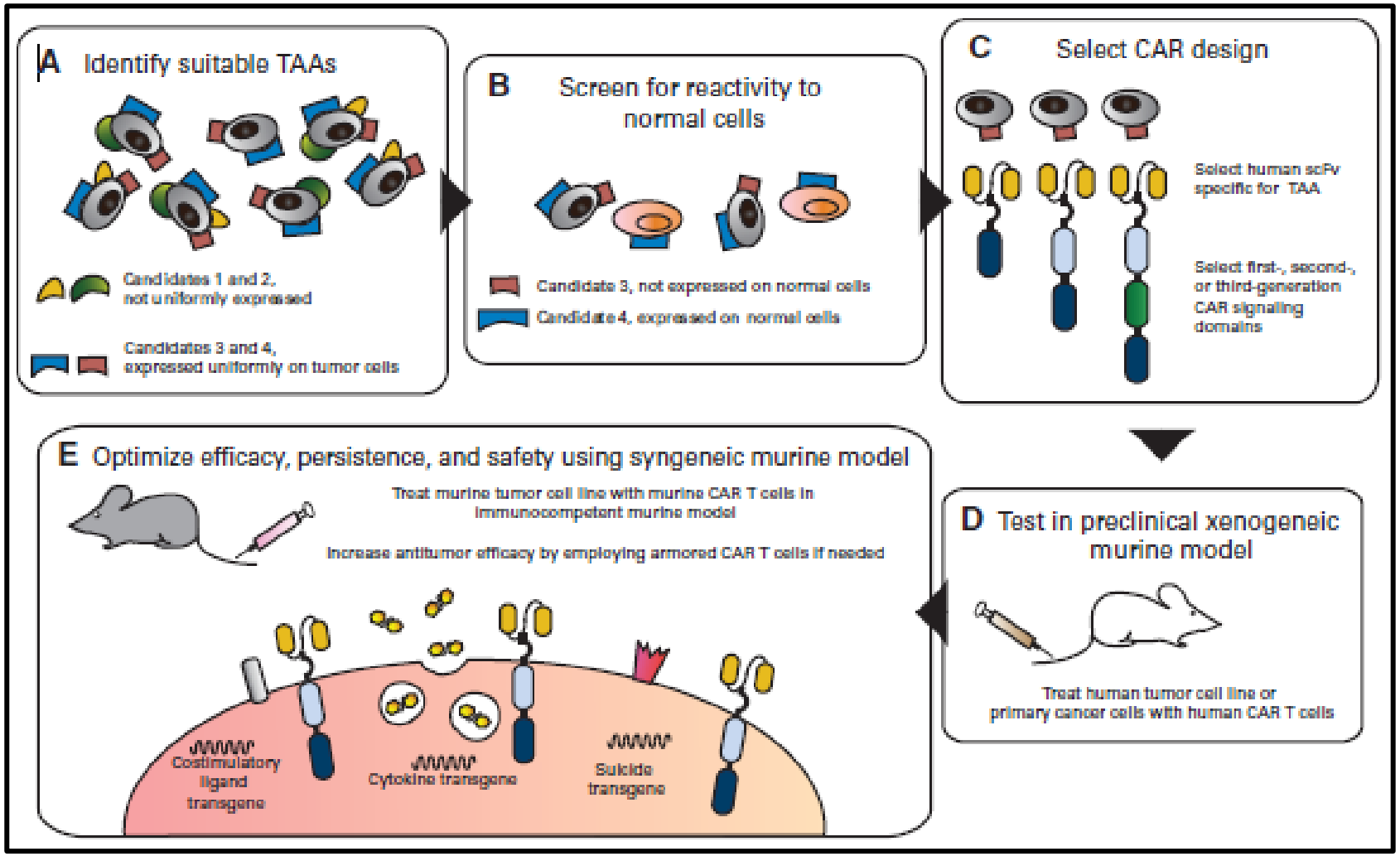
Classical academic research CAR-T cell production



Rossig, JCO 2023

CAR-T cells

Academic production : pre-clinical development



Curran, JCO 2015

CAR-T cells

Academic production : CAR-T manufacturing

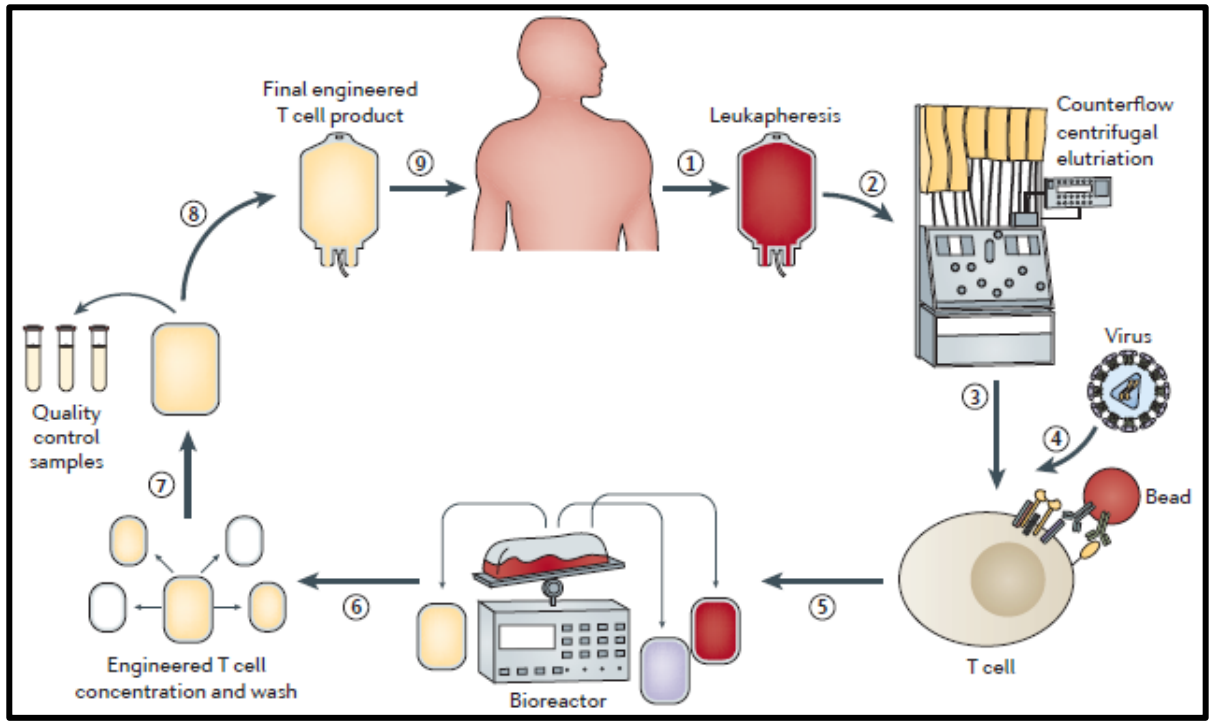


Table 8.3 CAR-T manufacturing methodology

	Potential methods	Timepoint
Step 1: T cell enrichment post-leukapheresis *(optional)*	Ficoll density gradient centrifugation; elutriation; immunomagnetic bead separation	Day 1
Step 2: T cell activation using synthetic antigen presenting technologies (CD3 +/− CD28) *(required)*	Soluble monoclonal antibodies; Para-magnetic anti-CD3/CD28 antibody coated beads; polymeric biodegradable CD3/28 incorporating nanomatrix (TransAct™)	Days 1, 2
Step 3: T cell stimulation *(required)*	IL-2, IL-7, and IL-15 in the culture medium (as per protocol) (Hoffmann et al. 2018; Gong et al. 2019)	From day 1 onwards
Step 4: Gene delivery/transduction with a retroviral or lentiviral CAR vector *(required)*	In some processes, retronectin or Vectofusin® is used to enhance transduction *(optional)*	Days 2, 3
Step 5: T cell expansion *(required)*	T-flasks, plates or culture bags; bioreactors, e.g., G-Rex™ flask (Wilson Wolf Manufacturing); Xuri WAVE™ Bioreactor (GE Life Systems); CliniMACS Prodigy™ (Miltenyi BioTec)	Days 3, 4 and onwards
Step 6: T cell harvest and cryopreservation *(required)*	The cryopreservation methodology often mirrors processes defined for haematopoietic cells. Methods include passive freezing (−80 °C freezer) and controlled-rate freezing	Day 8 onwards
Step 7: CAR-T cell quality assurance control and release testing	In-process and end of process controls are taken to ensure the product complies with release criteria specifications	Day 8 onwards

Fesnak, Nat Rev 2016

Delgado, EBMT/EHA CAR-T Cell Handbook

CAR-T cells

Academic production : QC

Vector production

Table 8.1 Quality control for the HEK293T master cell bank

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Presence of adherent cells with thin extensions
Sterility	Microbial growth	Sterile
Mycoplasma	PCR	Absent
Adventitious viruses	PCR	Absent
Karyotype	G-band staining	Informative
Cell viability (%) after thawing	Neubauer cell counting with trypan blue exclusion	>70%

Table 8.2 Quality control for GMP-grade virus production

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Yellowish liquid solution
Viral titre	Limiting dilution	$>3.75 \times 10^7$ TU/mL
Sterility	Microbial growth	Sterile
Mycoplasma	PCR	Absent
Identity	PCR	Positive
Replication-competent lentivirus	Real-time PCR	Absent

CAR-T cell production

Table 8.4 Quality control of CAR-T cell biology and microbiology

Parameter	Method	Acceptance criteria
Appearance	Visual inspection	Cloudy liquid solution
CAR+ cells (%)[a]	Flow cytometry	>20%
CD3+ cells (%)	Flow cytometry	>70%
Cell viability (%)	Neubauer cell counting with trypan blue exclusion[b]	>70%
Sterility	Microbial growth E. Ph. 2.6.1	Sterile from bacteria/fungi
Mycoplasma	PCR[c]	Absent
Endotoxin	Chromogenic assay	<0.5 EU/mL
Optional/R&D		
CAR/CD45RA/CCR7 For detection of TE/ TEM/TEMRA/TCM/TN subpopulations	Flow cytometry	A high proportion of immature T cells is desirable for a long-lasting CAR-T cell effect in the patient
Cytotoxic potency	Cr-51 release assays in tumour CAR-T cell co-culture, assessed by flow cytometry	>40% killing at an effector/target ratio of 10:1 (or higher ratio) in a 4-h assay
Adventitious viruses	PCR	Absent
Number of transgene copies/cell	Real-time PCR (Kunz et al. 2019; Schubert et al. 2020)	<10 (range <7–15!) copies/cell[d]

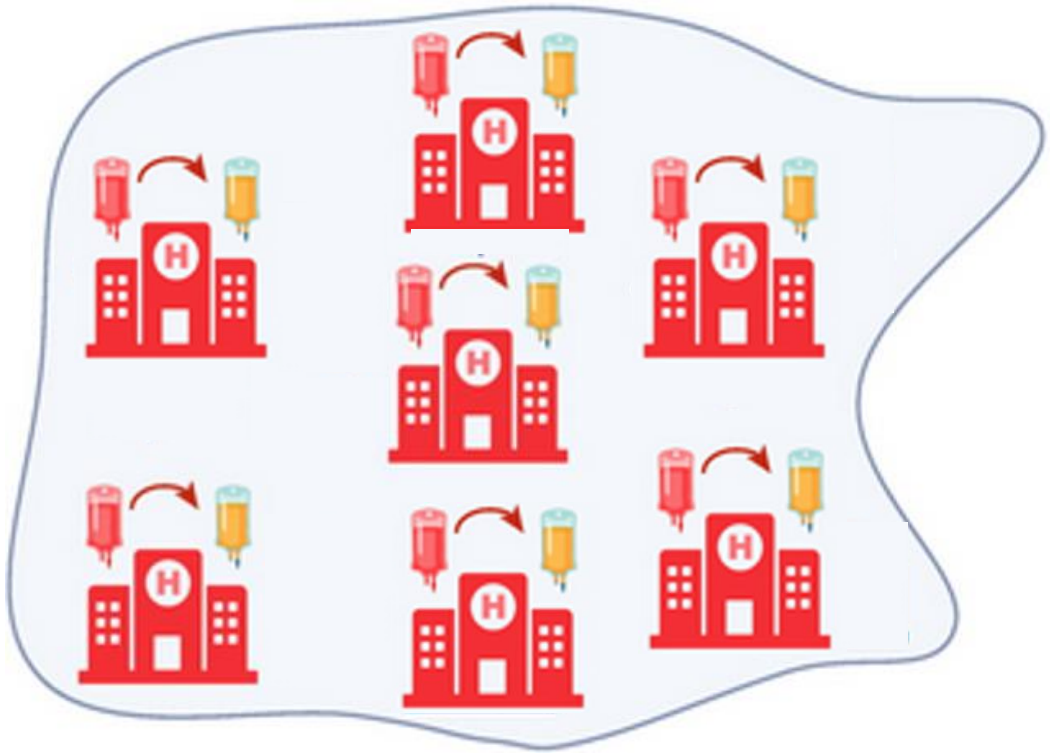
[a]Automated cell counters, such as Luna™, are highly recommended
[b]Highly specific detection reagents (e.g., the Miltenyi Detection Reagent™) are strongly advised to distinguish CAR-T cells from the negative fraction
[c]European standards stipulate PCR methodology, in contrast to US regulations, which require serology
[d]Differs between countries and products

Delgado, EBMT/EHA CAR-T Cell Handbook

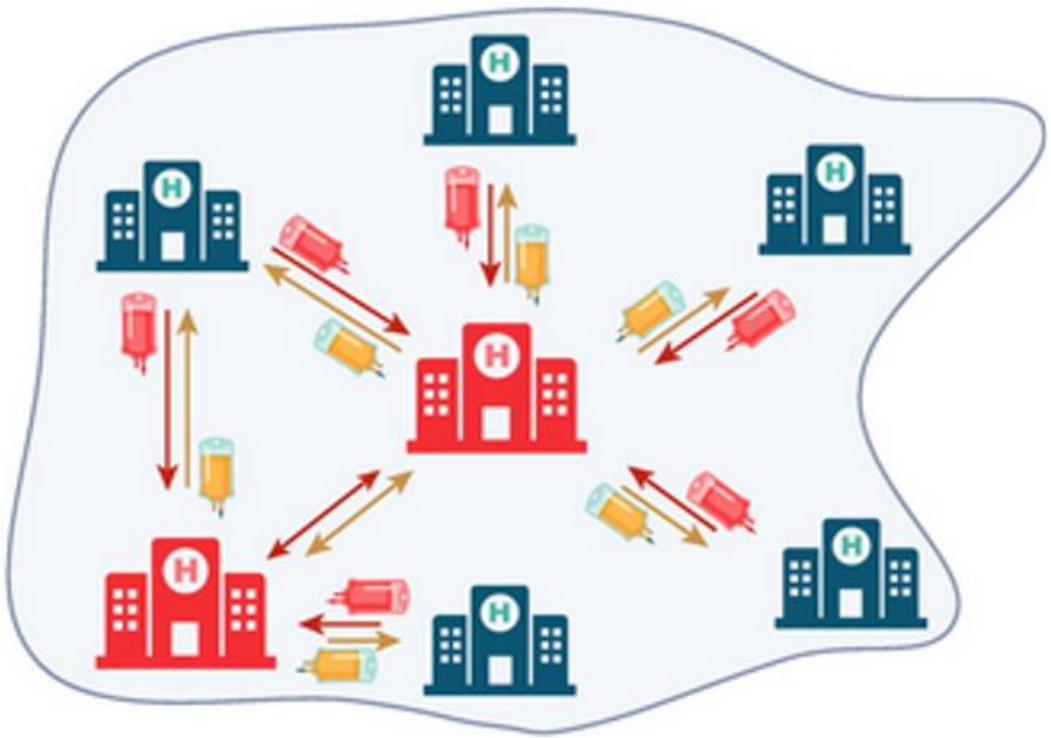
CAR-T cells

Point-of-care & decentralized CAR-T cell production

Point-of-care



Decentralized

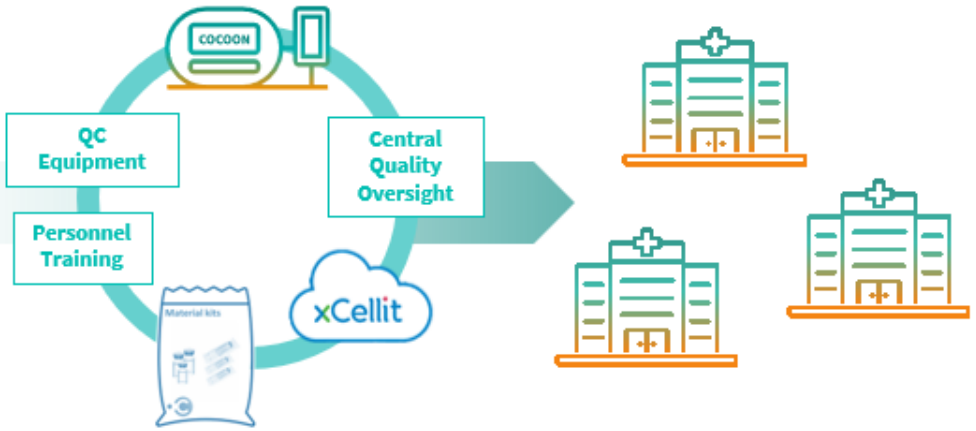


Rossig, JCO 2023

CAR-T cells

Galapagos decentralized production

Enabling scalable and consistent decentralized production



- ✓ Consistency by design
- ✓ Globally scalable
- ✓ 24/7 technical support
- ✓ Centrally supplied equipment / material kits

GMP production at a compliant manufacturing facility located at the clinic premises or in close proximity to the clinic

Clinical trials

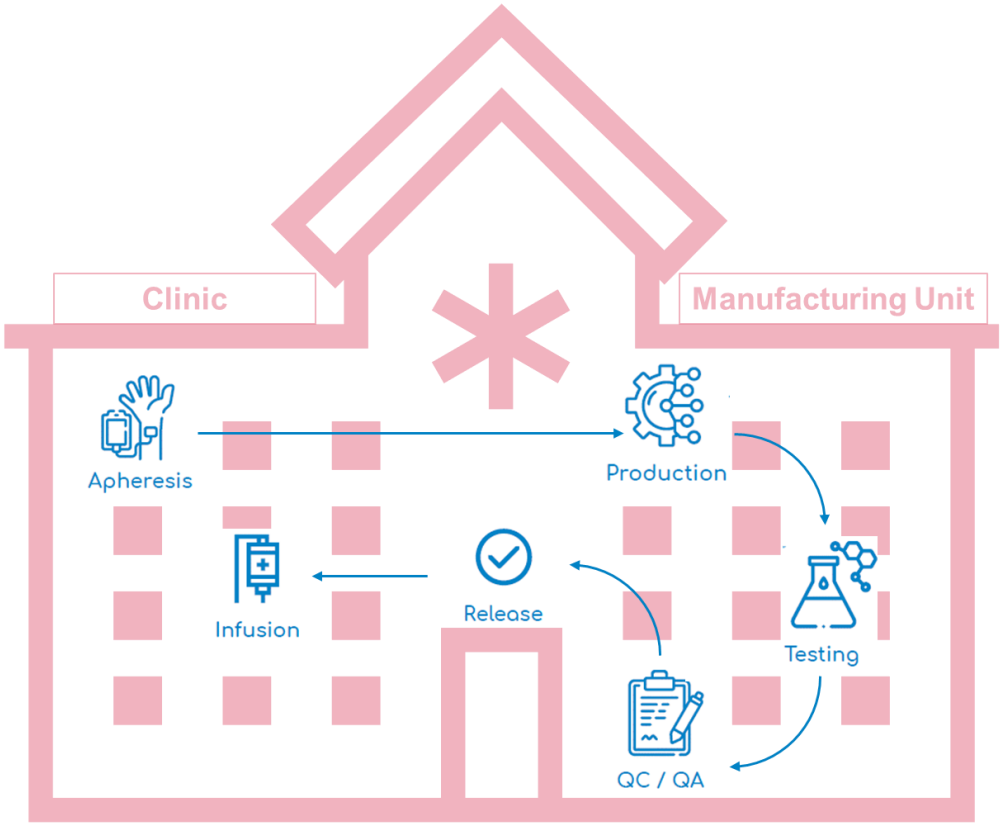
- **NHL : Atalanta-1**
- **CLL / RT : Euplagia-1**
- **MM : Papilio-1**

CAR-T cells

Galapagos decentralized production

Manufacturer
- Study Sponsor
- Real-time data capture
- Product responsibility

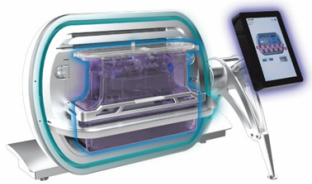
xCellit data platform



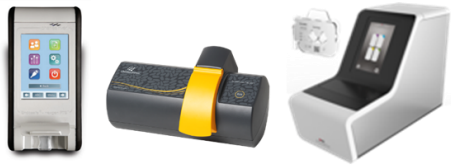
Site selection:
- Clinical CAR-T experience
- Cleanroom facility (class B-C)
- Operators

Materials kits

Tech transfer & support:
- Cocoon instruments
- QC instruments
- Materials kits
- Procedures & training
- Service & support



Cocoon



Rapid QC instruments

CAR-T cells

Production qualification for phase I-II

Production

QC

Tech transfer
Training
Integration into QM

3 media simulations (asepsis)

1 x/yr each operator
2 x/yr each process

Production QC

1 engineering run

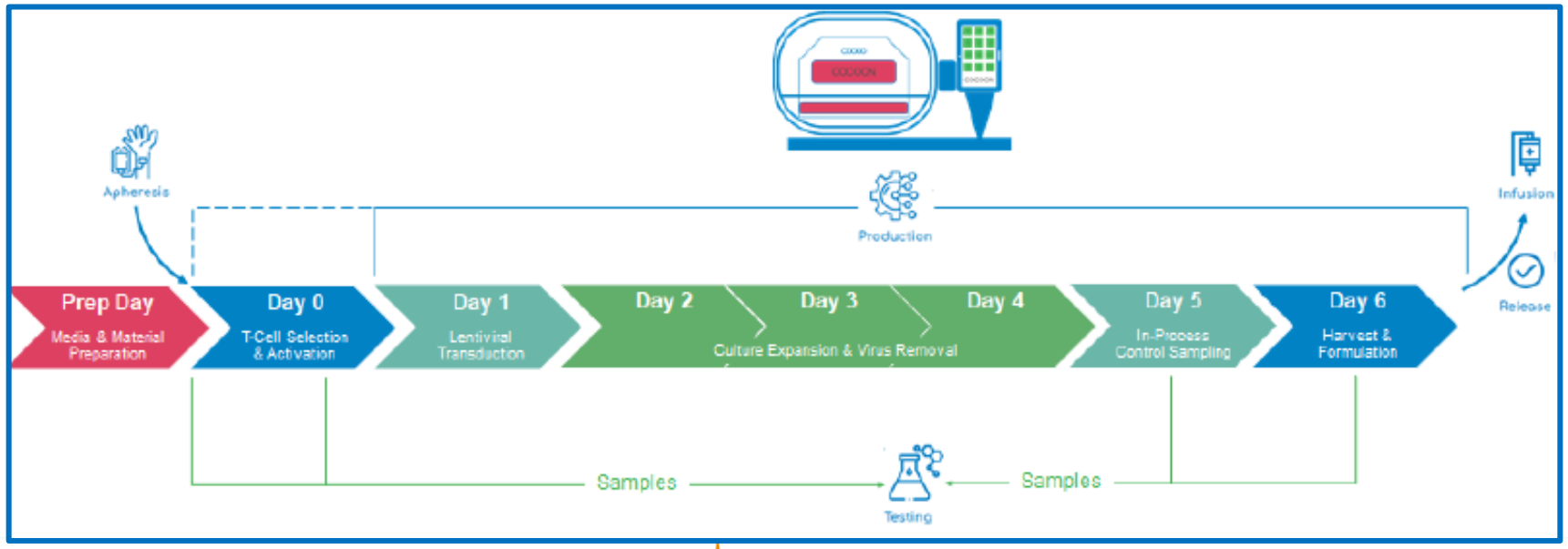
Production QC

3 qualification runs

Process qualification if all within specifications !

CAR-T cells

Galapagos decentralized production & patient journey



DECENTRALIZED MANUFACTURING

7 DAYS

Screening

Leukapheresis

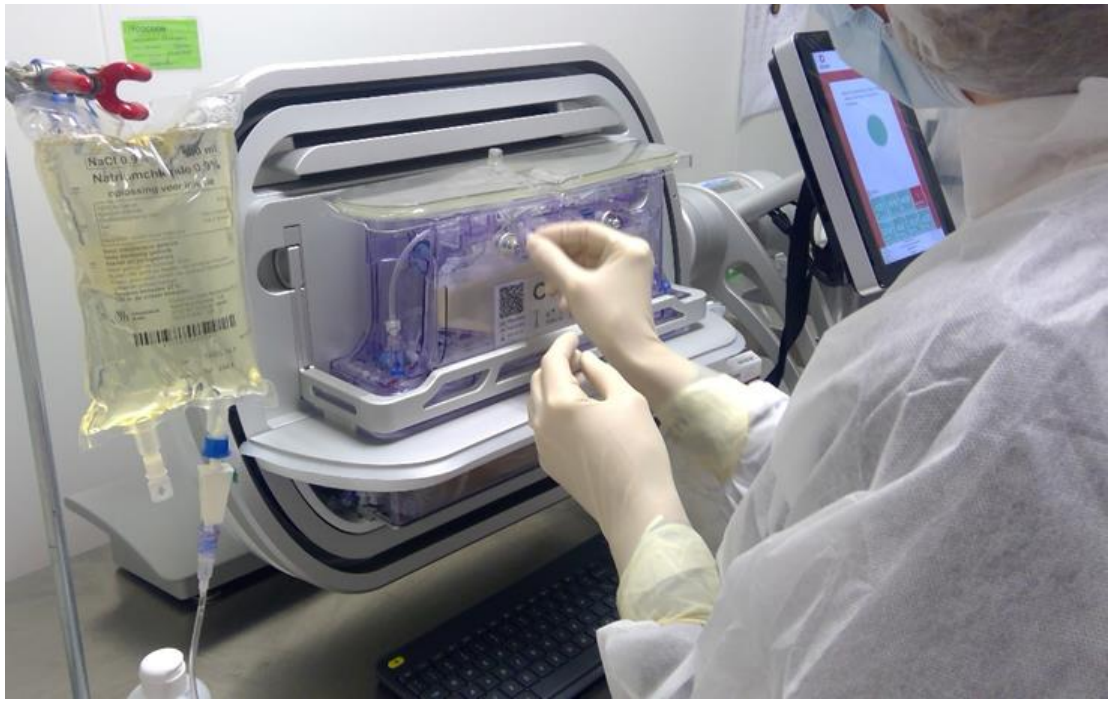
PATIENT CONDITIONING

CAR-T single fresh infusion

Follow-up visits

CAR-T CELLS

Production on Cocoon automated platform



CAR-T CELL THERAPY

CLINICAL TRIALS
NHL : ATALANTA

Seven-Day Vein-to-Vein Point-of-Care Manufactured CD19 CAR T Cells (GLPG5101) in R/R NHL: Results from the Phase 1/2 ATALANTA-1 Trial

MJ Kersten *et al*

Amsterdam, Leiden, Antwerp, Liège

Galapagos

EHA 2024

ATALANTA-1 Study Design and Objectives

Key eligibility criteria

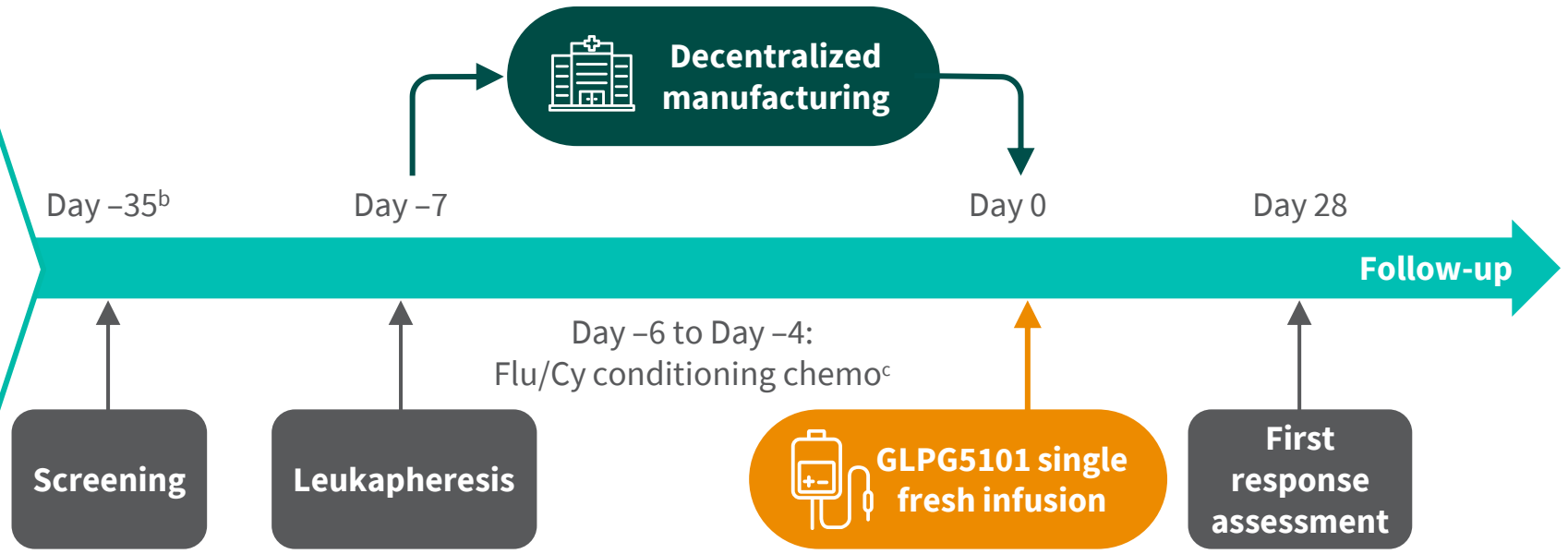
- No prior CD19-targeted therapies

Phase 1 dose escalation:

- DLBCL
  - Primary refractory or first relapse
- FL, MZL, MCL
  - Relapsed or refractory after two prior treatments

Phase 2 expansion cohorts:

- DLBCL, HR DLBCL,[a] FL + MZL, MCL, Burkitt lymphoma, PCNSL



Phase 1 primary objectives:
Safety
Determination of a RP2D

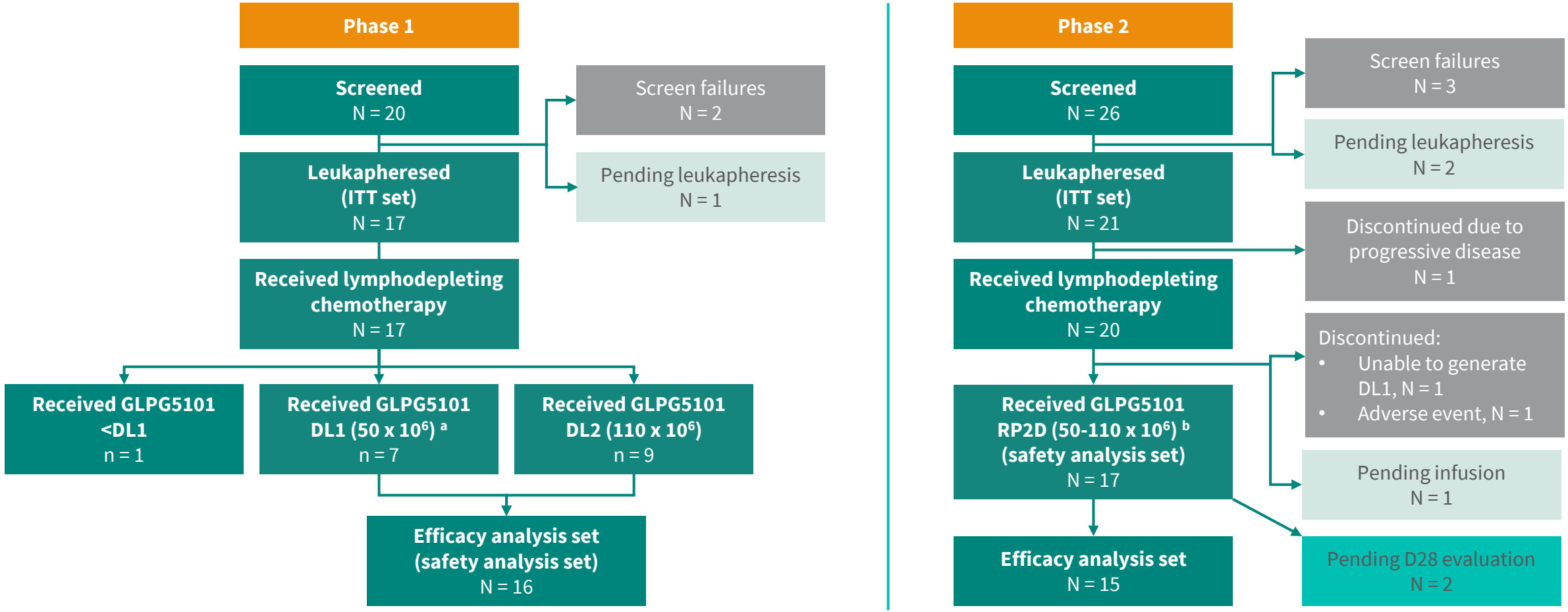
Phase 2 primary objective:
Efficacy (ORR)

Phase 1/2 secondary objectives:
Safety
Efficacy (CRR, DoR, MRD-, PFS, OS)
Pharmacokinetics and pharmacodynamics
Feasibility of decentralized manufacturing

[a]IPI 3–5 or double/triple-hit lymphoma. [b]Screening could take place up to a maximum of 28 days prior to leukapheresis. [c]Conditioning chemotherapy: fludarabine IV (30 mg/m^2/day); cyclophosphamide IV (300 mg/m^2/day).
Cy, cyclophosphamide; FL, follicular lymphoma; Flu, fludarabine; (HR) DLBCL, (high-risk) diffuse large B-cell lymphoma; IPI, international prognostic index; IV, intravenous; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; ORR, objective response rate; PCNSL, primary central nervous system lymphoma; RP2D, recommended Phase 2 dose

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Patient Disposition



[a]Includes 3 patients who received DL1 instead of planned DL2, due to lower manufacturing yield.
[b]Sixteen patients received RP2D; one patient received <RP2D.
DL, dose level; ITT, intention-to-treat; RP2D, recommended Phase 2 dose

Data cutoff: December 20, 2023.

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Decentralized Manufacturing

Enabling fresh product infusion with a 7-day vein-to-vein time

Median vein-to-vein time for product was **7 days** (range 7–13)

7 days
n = 30 (88%)

8 days
n = 2 (6%)

13 days
n = 2 (6%)

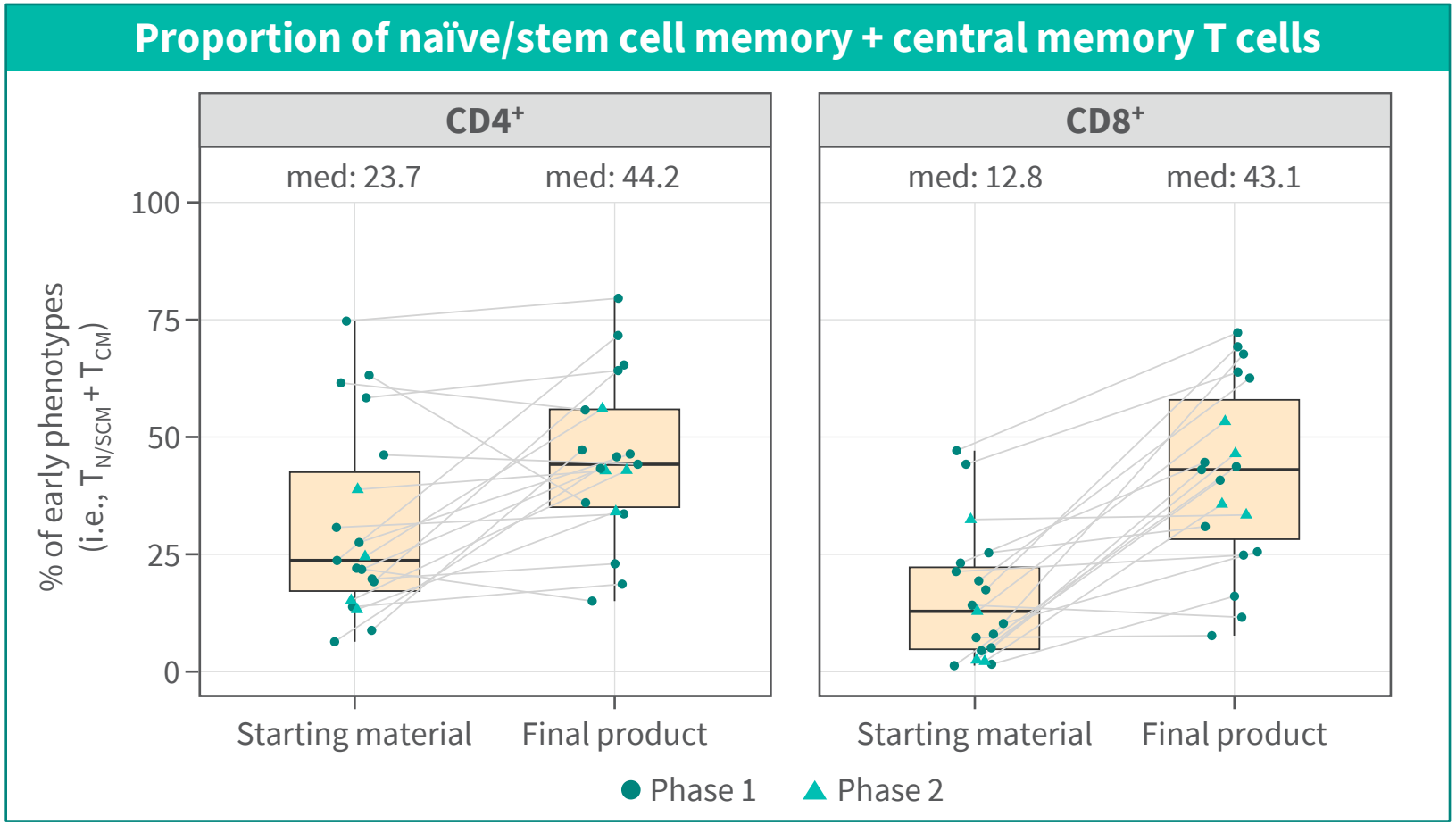
- Short vein-to-vein time eliminated the need for bridging therapy
- GLPG5101 was administered as a **fresh product** to 32/34 (94%) patients
  - ➢ Two patients received a cryopreserved product (vein-to-vein time 13 days)

Data cutoff: December 20, 2023.

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Product Characterization

Proportion of early phenotypes of CD4⁺ and CD8⁺ CAR T cells was increased in the final product compared with the starting material



Early phenotypes of CD4+ & CD8+ CAR T cells

=

Naïve/stem cell memory T cells ($CD45RO^{-}CD197^{+}$ $T_{N/SCM}$)

+

Central memory T cells ($CD45RO^{+}CD197^{+}$ T_{CM})

Exploratory flow cytometry analysis of T-cell subsets in the apheresis starting material and final product (gated on CAR⁺ T cells for final product) for paired patient samples (N=19).

CAR, chimeric antigen receptor; med, median; T_{CM}, central memory T cells; $T_{N/SCM}$, naïve/stem cell memory T cells

Data cutoff: September 01, 2023.

Adapted from presentation at EBMT 50th Annual Meeting: April 14–17, 2024; Glasgow, UK

Demographics and Baseline Characteristics

High-risk and heavily pretreated patients were included

	Phase 1 (DL1 & 2)	Phase 2
	All patients N = 16	All patients N = 17
Age, median (range), years	**65 (25–77)**	**67 (45–81)**
Male, n (%)	**12 (75)**	**9 (53)**
NHL subtype, n (%)[a]		
DLBCL	**9 (56)**	**0**
MCL[b]	**3 (19)**	**4 (24)**
FL	**3 (19)**	**12 (71)**
MZL	**1 (6)**	**1 (6)**
IPI/MIPI/FLIPI score at screening, high risk, n (%)	**6 (38)**	**11 (65)**
ECOG PS at baseline, n (%)		
0	**6 (38)**	**8 (47)**
1	**9 (56)**	**6 (35)**
2	**1 (6)**	**3 (18)**
Previous therapies, median (range)	**3 (1–7)**	**3 (2–11)**
Ann Arbor disease stage, n (%)		
II	**1 (6)**	**4 (24)**
III–IV	**15 (94)**	**13 (76)**

[a]Sum of percentages may be >100 due to rounding. [b]Two patients with MCL were not included in the Phase 2 efficacy analysis set as the first response assessment data were not available at data cutoff.

Data cutoff: December 20, 2023.

DLBCL, diffuse large B-cell lymphoma; ECOG PS, Eastern Cooperative Oncology Group performance status; FL, follicular lymphoma; (M, FL)IPI, (MCL, FL) international prognostic index; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Safety: TEAEs

Most Grade ≥3 TEAEs were hematological

TEAEs up to 14 weeks after infusion	Phase 1 (DL1 & 2) All patients N = 16	Phase 2 All patients N = 17
Any TEAE, n (%)	**16 (100)**	**17 (100)**
Any GLPG5101-related TEAE, n (%)	**16 (100)**	**14 (82)**
Serious TEAE, n (%)	**5 (31)**	**3 (18)**
TEAE leading to death, n (%)	**1 (6)**	**0**
Any Grade ≥3 TEAE, n (%)	**16 (100)**	**14 (82)**
Hematological Grade ≥3 TEAEs, n (%)		
Neutropenia[a]	**15 (94)**	**12 (71)**
Anemia[b]	**6 (38)**	**1 (6)**
Lymphopenia[c]	**5 (31)**	**3 (18)**
Thrombocytopenia[d]	**4 (25)**	**4 (24)**
Leukopenia[e]	**6 (38)**	**5 (29)**
Other Grade ≥3 TEAEs in ≥2 patients[f], n (%)		
Pyrexia	**2 (13)**	**1 (6)**
Pleural effusion	**2 (13)**	**0**

[a]Includes neutropenia/neutrophil count decreased. [b]Includes anemia/hemoglobin decreased. [c]Includes lymphopenia/lymphocyte count decreased. [d]Includes thrombocytopenia/platelet count decreased. [e]Includes leukopenia/white blood cell count decreased. [f]In either the Phase 1 or Phase 2 total population. **Data cutoff: December 20, 2023.**
TEAE, treatment-emergent adverse event

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Safety: AESIs and Deaths

The vast majority of CRS and ICANS events were low-grade

AESIs up to 14 weeks after infusion	Phase 1 (DL1 & 2) All patients N = 16	Phase 2 All patients N = 17
CRS (n, %)	7 (44)	5 (29)
Grade 1	2 (13)	4 (24)
Grade 2	3 (19)	1 (6)
Grade 3	2 (13)	0
ICANS (n, %)	6 (38)	1 (6)
Grade 1	6 (38)	0
Grade 2	0	0
Grade 3	0	1 (6)
Infections, Grade ≥3 (n, %)	1 (6)	0
Prolonged cytopenia,[a] Grade ≥3, (n,%) 30 days after infusion[b] 60 days after infusion[c]	 7 (47) 4 (27)	 5 (36) 3 (27)
Hemophagocytic lymphohistiocytosis, any grade (n, %)	1 (6)	0

CRS and ICANS

Two cases of Grade 3 CRS in Phase 1

One case of Grade 3 ICANS in Phase 2

Deaths during treatment (up to 14 wks after infusion)

Intra-abdominal hemorrhage, caused by DIC
Phase 1, DL2[e]

Respiratory distress, caused by disease progression & respiratory infection
Phase 1, <DL1[d]

Deaths post-treatment period[g]:

Escherichia sepsis
Phase 1, DL2[e,f]

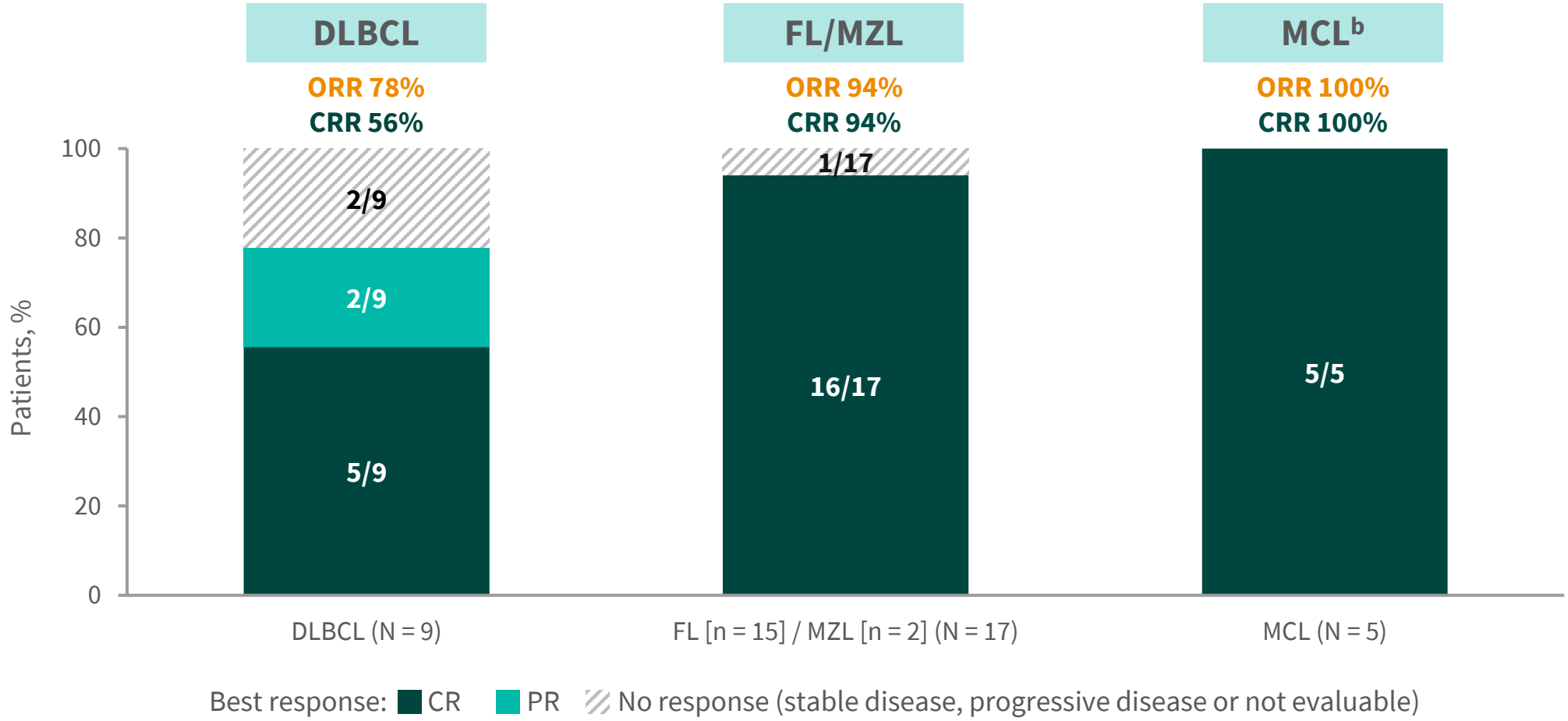
Data cutoff: December 20, 2023.

[a]Includes all events related to neutropenia, thrombocytopenia, anemia and lymphopenia. [b]Data available for 15 patients in Phase 1 and 14 patients in Phase 2. [c]Data available for 15 patients in Phase 1 and 11 patients in Phase 2. [d]DL1 = 50×10^6 CAR+ T cells. [e]DL2 = 110×10^6 CAR+ T cells. [f]Reported >6 months post-infusion, in a patient with hypogammaglobinemia. AESI, adverse event of special interest; CRS, cytokine release syndrome; DIC, disseminated intravascular coagulation; DL, dose level; ICANS, immune effector cell-associated neurotoxicity syndrome

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Efficacy: Pooled Phase 1/2 Results

High OR and CR rates were observed[a]

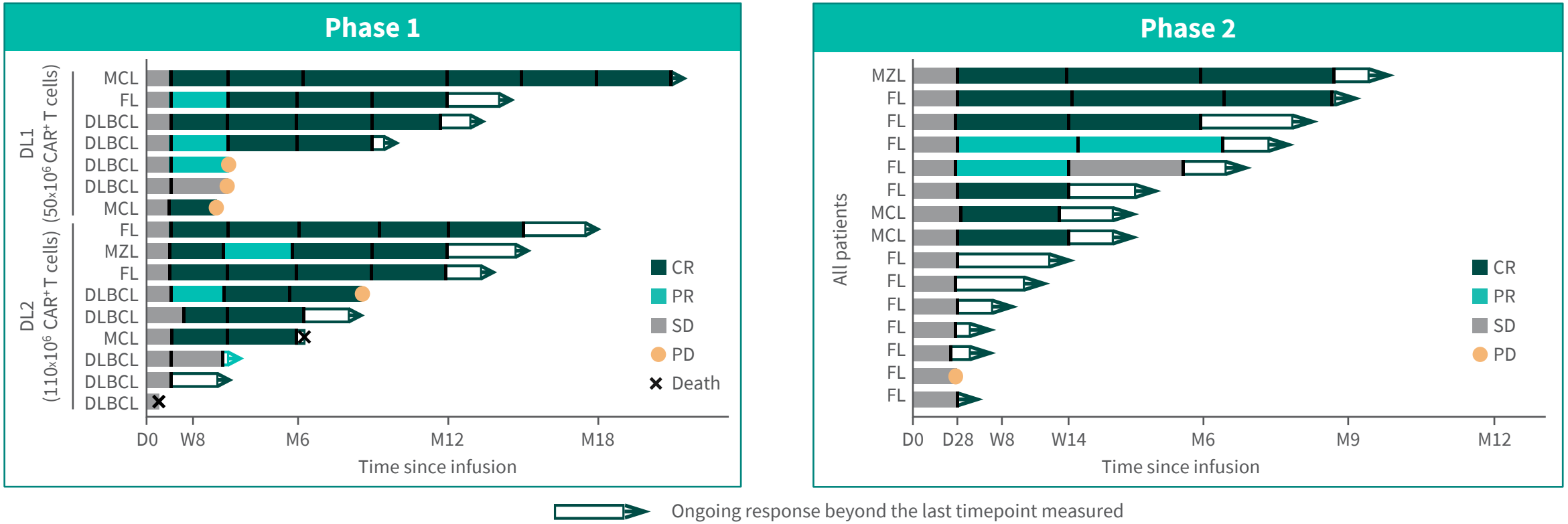


[a]Best response at any time after infusion. [b]Two patients with MCL were not included in the Phase 2 efficacy analysis set as the first response assessment data were not available at data cutoff. **Data cutoff: December 20, 2023**.
CR, complete response; CRR, complete response rate; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; OR, objective response; ORR, objective response rate; PR, partial response

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Efficacy: Response Over Time

Durable responses were observed



Ongoing response beyond the last timepoint measured

In Phase 1, 10/14 (71%) patients had an ongoing response

Median follow-up in study : **13.1 months (range 0.5–21.0)**

In Phase 2, 14/14 (100%) patients had an ongoing response[a]

Median follow-up in study : **4.2 months (range 1.0–9.4)**

[a]Two patients with MCL were not included in the Phase 2 efficacy analysis set as the first response assessment data were not available at data cutoff. **Data cutoff: December 20, 2023.**

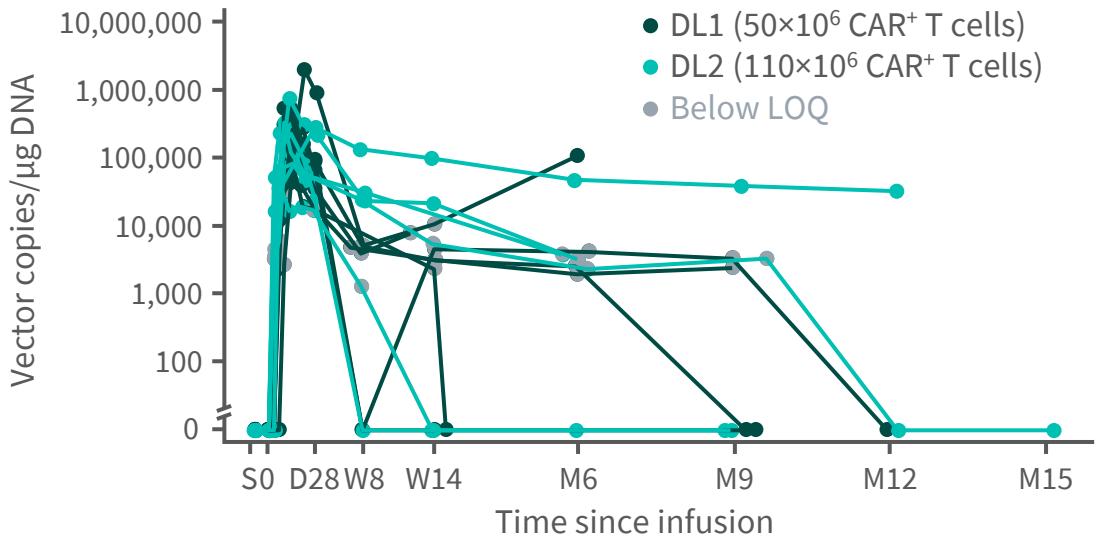
CAR, chimeric antigen receptor; CR, complete response; D, Day; DL, dose level; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; M, Month; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PD, progressive disease; PR, partial response; SD, stable disease; W, Week

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

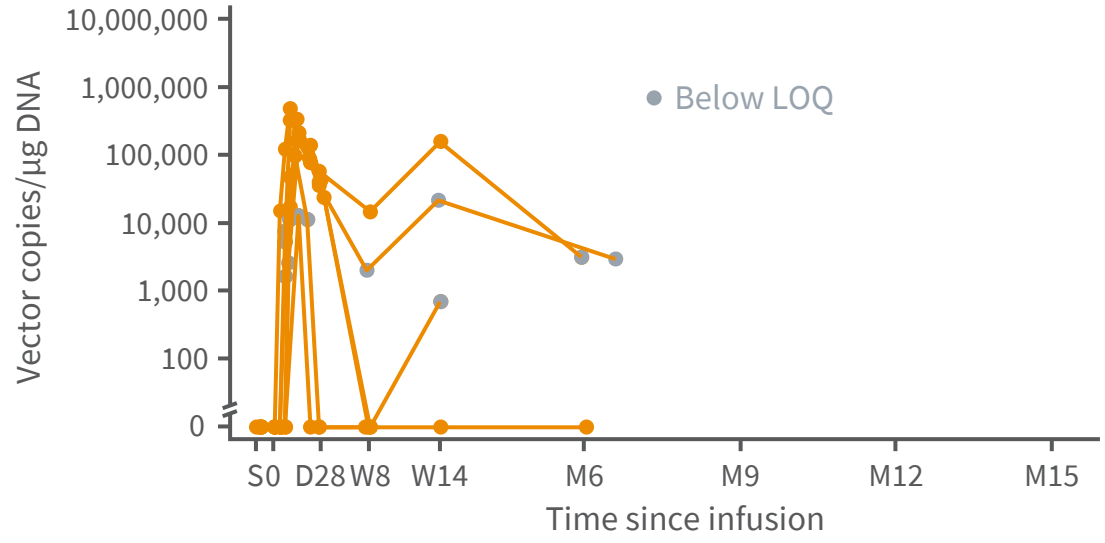
Cellular Kinetics

GLPG5101 demonstrated the ability for durable persistence and robust expansion across doses

Phase 1



Phase 2



11/15 (73%) patients had detectable GLPG5101 in peripheral blood at Week 14 post-infusion

Persisting CAR T cells could be detected up to 12 months post-infusion

Quantification of GLPG5101 in peripheral blood by qPCR. LOQ: 1,000 vector copies. **Data cutoff: December 20, 2023.**

CAR, chimeric antigen receptor; D, Day; DL, dose level; LOQ, limit of quantification; M, Month; S, screening; W, Week

Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

Conclusions and Study Updates

- Data from **33 patients with relapsed/refractory NHL** enrolled in the ongoing Phase 1/2 ATALANTA-1 study demonstrate that **decentralized CAR T-cell manufacturing with a short vein-to-vein time is feasible**
- GLPG5101 was administered as a **fresh and fit product** with a **median vein-to-vein time of 7 days**
- GLPG5101 demonstrated **robust *in vivo* expansion and durable persistence** post-infusion
- The **vast majority of CRS and ICANS events were Grade 1 or 2**; **two cases of Grade 3 CRS** and **one case of Grade 3 ICANS** were reported
- **High complete response rates were observed across indications** in this heavily pretreated population

Study updates

- The RP2D in FL, MZL and MCL is DL2 (110 (range 50-110) x 10^6 CAR+ T cells)
- Dose escalation in DLBCL is ongoing at DL3 (250 x 10^6 CAR+ T cells)
- Additional expansion cohorts of patients with BL[a] and PCNSL[a] will be treated at the RP2D for DLBCL

[a]After receiving two or more prior treatments.
BL, Burkitt lymphoma; DL, dose level; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PCNSL, primary central nervous system lymphoma RP2D, recommended Phase 2 dose; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; NHL, non-Hodgkin lymphoma

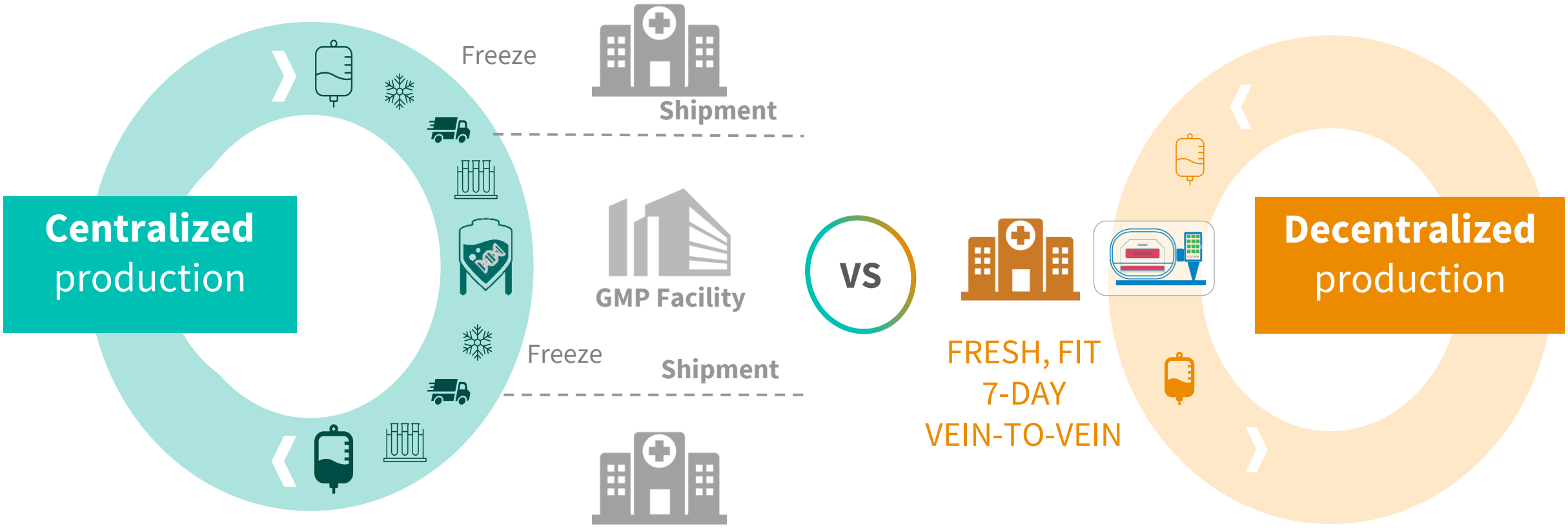
Adapted from presentation at EHA Hybrid Congress: 13–16 June, 2024; Madrid, Spain

CAR-T CELL THERAPY

DISCUSSION

CAR-T cells

Commercial vs decentralized production



CAR-T cells

Decentralized production : advantages

- **Potential advantages**
  - Fresh product
  - Short vein-to-vein time (7 days)
  - No need for bridging therapy
  - Simplified logistics
  - Potentially lower costs
  - Improved patient access

  - Close partnership between industry & hospital
  - Very motivating for Lab of Cell & Gene Therapy
  - Integrated production/QC & clinical use

CAR-T cells

Decentralized production : difficulties

- **Potential difficulties**
  - Requirement for academic GMP manufacturing & QC site : facilities, equipment, reagents, staff, QM system, accreditation by regulatory authorities
  - If ATMP comes from academic research :
    - Pre-clinical product development
    - Validated manufacturing & QC processes
    - Marketing authorization & pharmacovigilance
    - Funding
  - If ATMP comes from biotech company :
    - Tech transfer, training & validation at each site (production & QC)
    - Standardization across multiple sites (production & QC)
    - Quality agreement to define respective responsibilities
    - Financial agreement
  - Clinical trials (phase 1-2 vs phase 3) versus commercialization

CAR-T cells

Decentralized production : responsibilities

- **Research : clinical trials → sponsor**
- **Commercialization → biotech company vs production site ?**

Items

- **Management of reagents (other than viral vector) & equipment**
- **Manufacturing issues : out-of-specification, failure, change control**
- **QC issues : decentralized vs centralized, change control**
- **Patient issues : drop-out, unexpected AE, long-term pharmacovigilance**

→ Legal & financial responsibility

CAR-T cells

Decentralized production : cost structure

- **Biotech company = MA holder**
  - IP on reagents : viral vector…
  - IP on whole production & QC processes
  - Centralized data platform
  - Long-term pharmacovigilance & unexpected AE
- **Production site**
  - Facility building & maintenance
  - Staff recruitment & training
- **To be decided / shared**
  - Equipment purchase & maintenance
  - Management of reagents (other than the ones covered by Biotech IP)
  - Responsibilities for OOS, manufacturing failures, patient drop-out

THANK YOU

FOR YOUR ATTENTION !