# Cellule CD19-CAR T allogeniche da donatore: un approccio promettente per pazienti pediatrici con Leucemia Linfoblastica Acuta B-Cellulare altamente refrattaria

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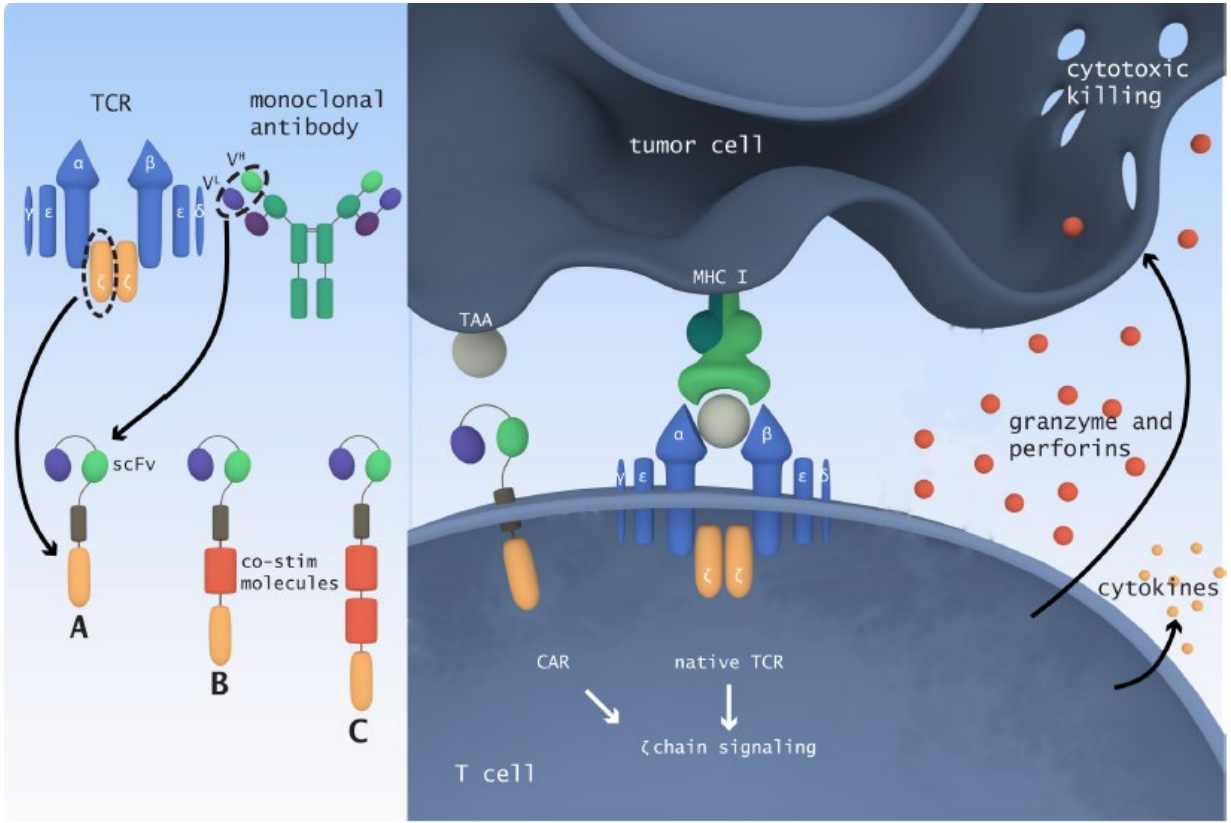
Bologna, 03/10/2023

XLVIII CONGRESSO NAZIONALE AIEOP

Bologna
2-4 Ottobre 2023

# Chimeric Antigen Receptor (CAR)



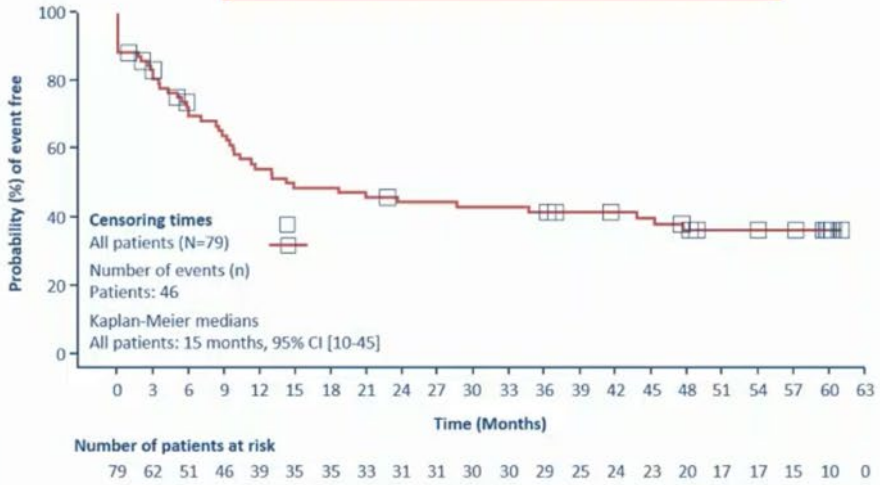

Hoyos et al. Haematologica 2012

# Kymriah: long-term outcome

## Median EFS was 15 Months

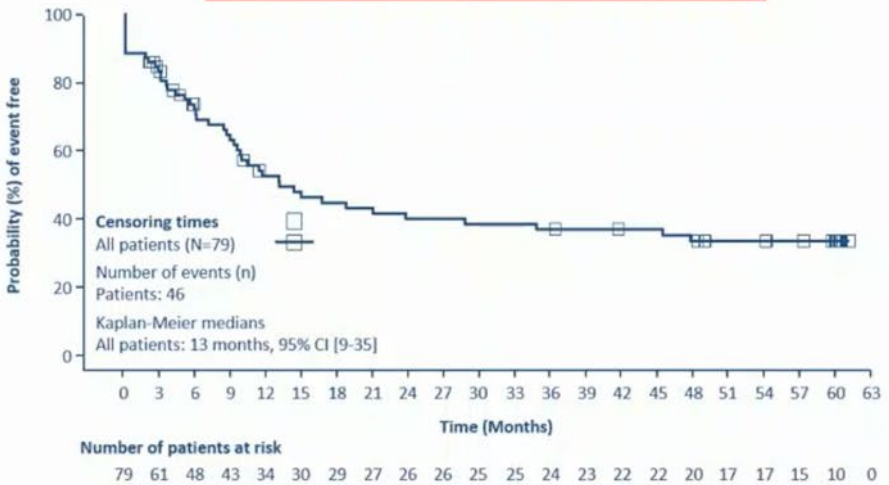
EFS Without Censoring for alloSCT

**5-year EFS: 36% (95% CI, 25%-47%)**




EFS With Censoring for alloSCT

**5-year EFS: 34% (95% CI, 23%-45%)**

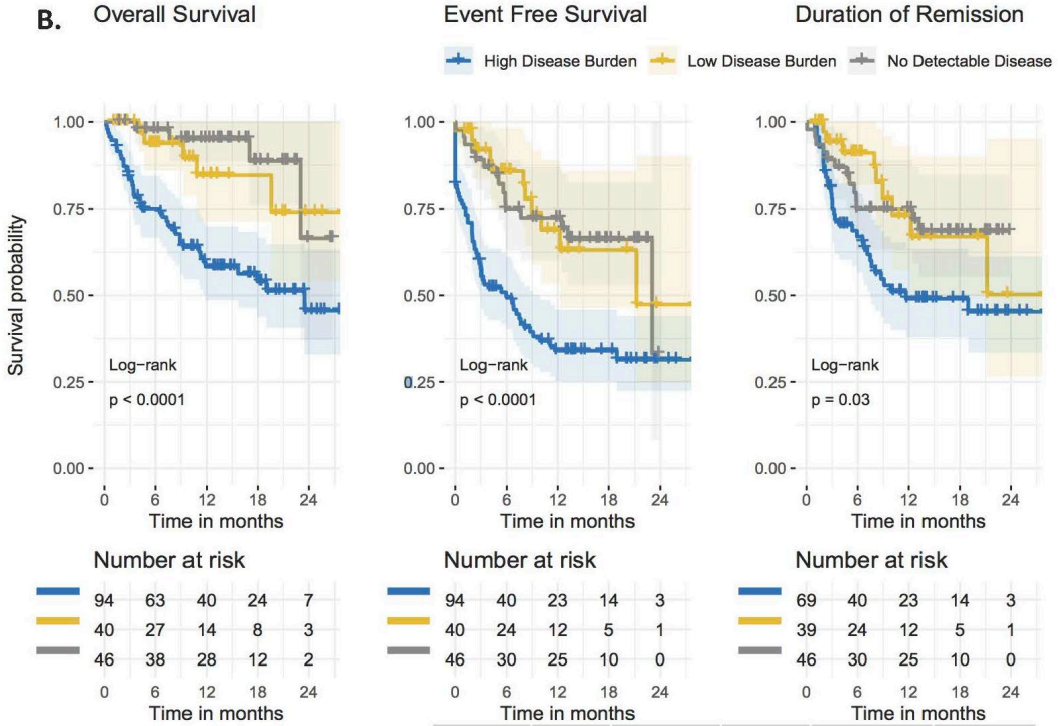



alloSCT, allogeneic stem cell transplantation; EFS, event-free survival; NE, not estimable.



Rives S, EHA 2022

# Real-world experience with Tisagenlecleucel



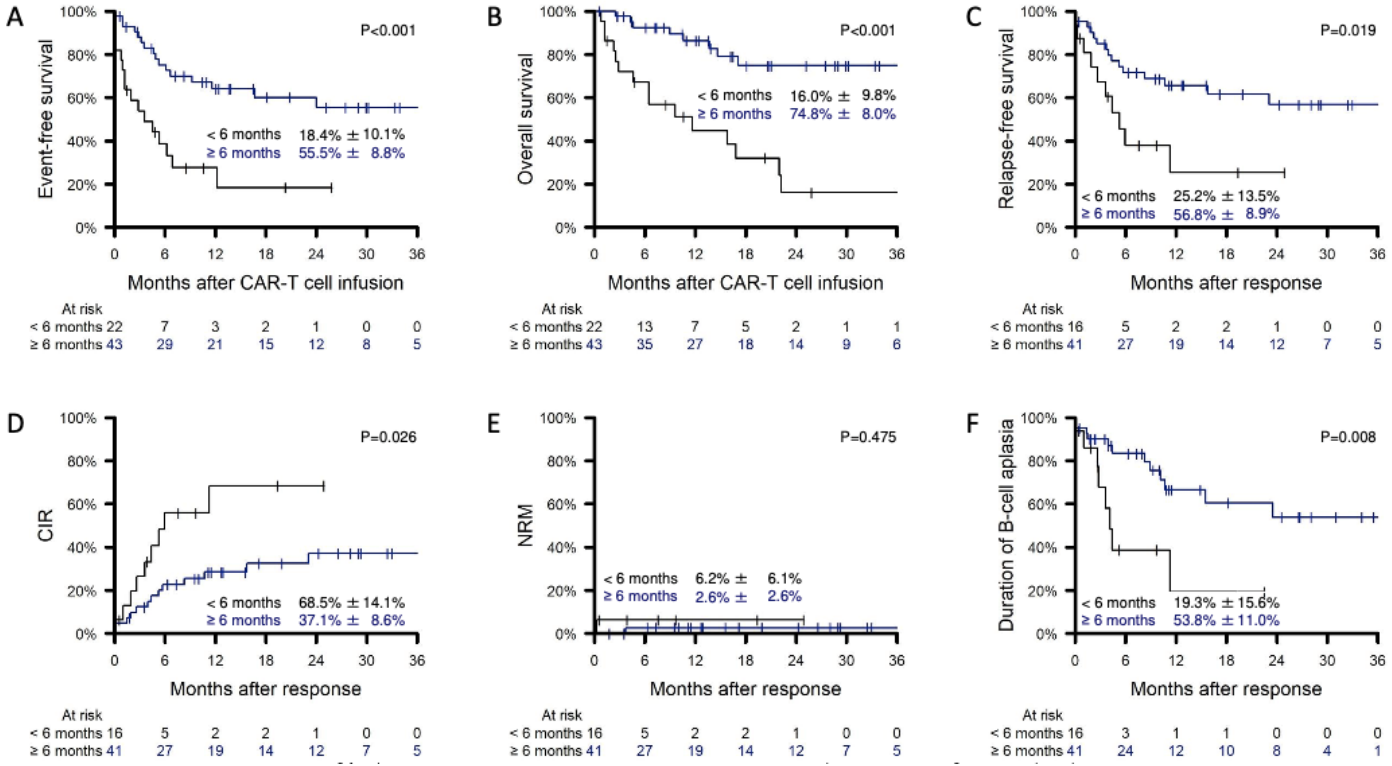

| | 6mo OS | 1y OS | 6mo EFS | 1y EFS | 6mo Drem | 1y Drem |
|---|---|---|---|---|---|---|
| *High Disease Burden* | 0.75 | 0.58 | 0.50 | 0.34 | 0.67 | 0.49 |
| *Low Disease Burden* | 0.94 | 0.85 | 0.86 | 0.69 | 0.91 | 0.73 |
| *No Detectable Disease* | 0.98 | 0.95 | 0.75 | 0.72 | 0.75 | 0.75 |

**High Disease Burden:**

- **>5% bone marrow lymphoblasts,**
- **peripheral blood lymphoblasts,**
- **CNS3 status**
- **non-CNS extramedullary (EM) site of disease**

Schultz L.M. et al., J Clin Oncol 2022

# Relapse < 6 months after HSCT is associated with a significantly worst outcome after tisagenlecleucel infusion




Bader P et al. Blood Adv 2023

# Determinants of efficacy of CAR T cells

**Vector integration**
Genomic integration site (impact of viral vector??)

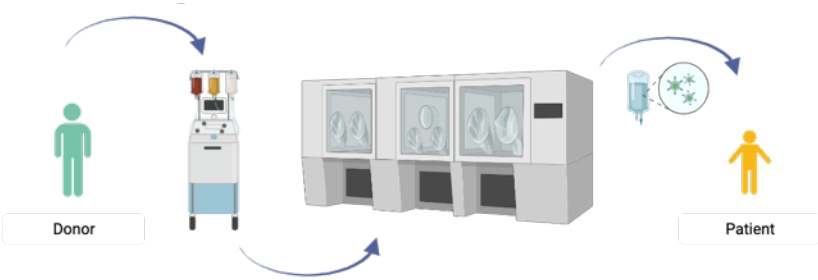
**B-cell/disease factors**
Low CD19 antigen load

**CAR T construct**
Costimulation (CD28 vs 4.1BB)

**Lymphodepletion**
Flu/Cy vs other

**T-cell factors**
- *Fitness* of T cells
- Initial T cell phenotype (CD4/CD8, early memory, exhaustion)

**Use of healthy, allogeneic donor**




Shah NN and Fry TJ. Nat Rev Clin Oncol 2019
Gardner R et al. Blood 2017

# Allogeneic, donor-derived CD19-CAR T cells: OPBG HE experience

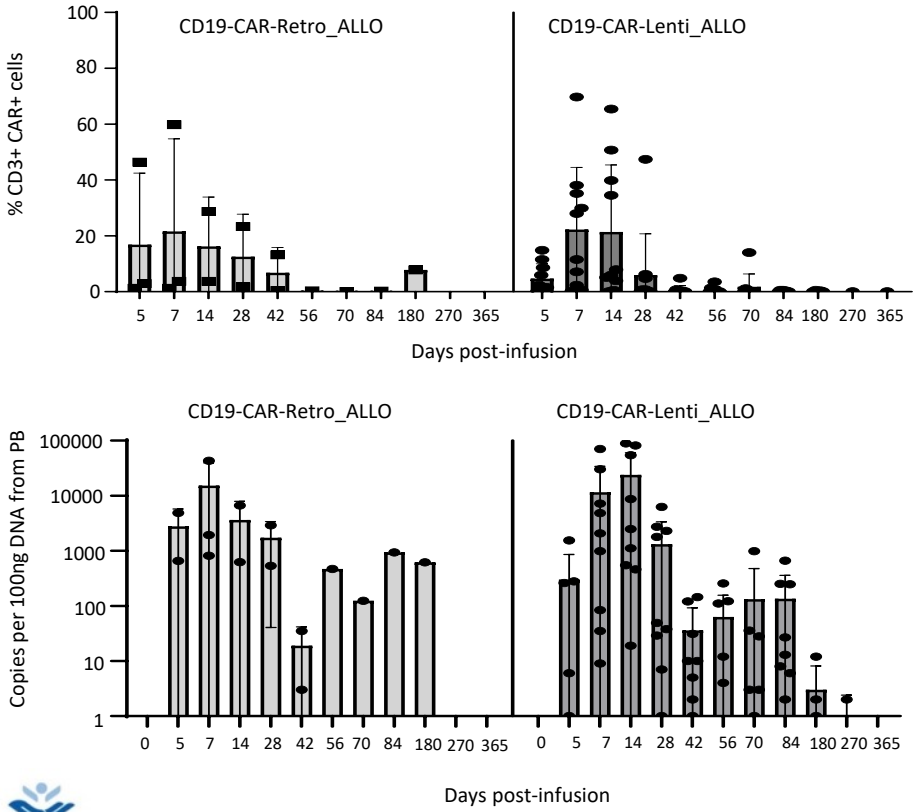
| Pt ID | Sex | Age (y) | Cytogenetic anomalies | Disease phase at infusion | Donor and HLA matching | CAR-T product | CAR⁺ T cells (x $10^6$ cells/kg) | CAR⁻ T-cells (x $10^6$ cells/kg) | Disease status at LD |
|---|---|---|---|---|---|---|---|---|---|
| Allo-CD19-CAR001 | F | 17 | iAmp21 | 2nd relapse, very early post-HSCT | MUD 10/10 | CD19-CAR-Retro_ALLO | 3.0 | 4.24 | BM (0,3%) |
| Allo-CD19-CAR002 | M | 21 | MEF2D/BCL9 | 1st refractory relapse | MFD | CD19-CAR-Retro_ALLO | 3.0 (PRE-HSCT) | 3.65 | BM (12%) + bone (>10 spots) + liver |
| Allo-CD19-CAR003 | M | 29 | KMT2A | 5th relapse | MFD | CD19-CAR-Retro_ALLO | 3.0 | 1.01 | Pelvic lymph nodes + CNS |
| CD19-LENTI-ALLO001 | M | 11 | TEL/AML1 | 4th relapse | MFD | CD19-CAR-Lenti_ALLO | 1.0 | 1.92 | BM (0,2%) + bone and kidney |
| CD19-LENTI-ALLO002 | M | 6 | None | 5th relapse (after 2 HSCTs) | Haplo | CD19-CAR-Lenti_ALLO | 2.0 | 1.49 | BM (83,7%) |
| CD19-LENTI-ALLO003 | M | 16 | None | 2nd relapse, very early post-HSCT | MFD | CD19-CAR-Lenti_ALLO | 2.0 | 4.17 | BM (0,2%) |
| CD19-LENTI-ALLO004 | M | 8 | IKAROS+ | 3rd relapse, after HSCT | MFD | CD19-CAR-Lenti_ALLO | 3.0 | 7.56 | BM (1,6%) |
| CD19-LENTI-ALLO005 | M | 7 | t(9;22) | 1st refractory relapse | MFD | CD19-CAR-Lenti_ALLO | 3.0 (PRE-HSCT) | 4.3 | BM (0,03%) |
| CD19-LENTI-ALLO006 | M | 17 | None | 2nd refractory relapse | Haplo | CD19-CAR-Lenti_ALLO | 3.0 | 4.21 | BM (0,01%) + bone |
| CD19-LENTI-ALLO007 | M | 4 | 47, XY (+21) | 1st refractory relapse | MFD | CD19-CAR-Lenti_ALLO | 3.0 | 3.15 | BM (0,02%) + ocular disease |
| CD19-LENTI-ALLO008 | F | 26 | None | 4th relapse (after HSCT and autologous CD19-CAR) | Haplo | CD19-CAR-Lenti_ALLO | 3.0 | 1.86 | BM (0,03%) + mammary gland + bone |
| CD19-LENTI-ALLO009 | M | 9 | None | 1st refractory relapse | MFD | CD19-CAR-Lenti_ALLO | 3.0 | 5.33 | BM (55%) |
| CD19-LENTI-ALLO010 | F | 33 | KMT2-A | 1st relapse after HSCT, very early (2 months) | MFD | CD19-CAR-Lenti_ALLO | 3.0 | 5.33 | BM (35%) |
| CD19-LENTI-ALLO011 | F | 34 | None | 3rd relapse, after HSCT | MFD | CD19-CAR-Lenti_ALLO | 3.0 | 3.74 | CNS3 |
| CD19-LENTI-ALLO012 | M | 27 | TCF3/PBX1 | 2nd relapse, early post-HSCT | MUD (9/10) | CD19-CAR-Lenti_ALLO | 3.0 | 4.19 | BM (14%), CNS2 |
| CD19-LENTI-ALLO014 | M | 6 | None | 2nd relapse, post-HSCT | Haplo | CD19-CAR-Lenti_ALLO | 3.0 | 4.25 | BM (4X10-3) + kidneys |

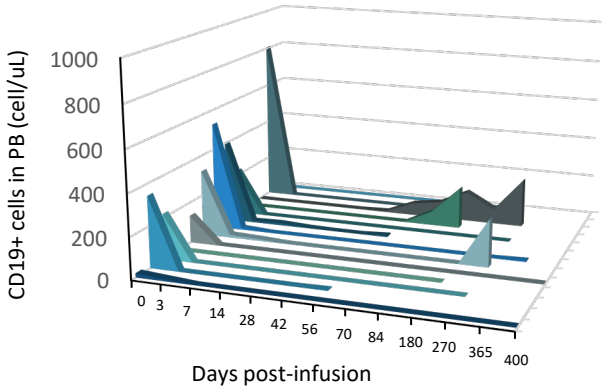
# Allogeneic, donor-derived CD19-CAR T products

| | CD19-CAR-Retro_ALLO | CD19-CAR-Lenti_ALLO |
|---|---|---|
| **Viral platform** | Retrovirus | Lentivirus |
| **Production system** | Manual, using bioreactors | Automated (CliniMACS Prodigy®) |
| **Starting material** | Cryopreserved, bulky apheresis (1.5 x $10^9$ total WBC) | Fresh apheresis (0.75-1.5 x $10^9$ total WBC) |
| | | CD4/CD8 enriched cells (20-200 x $10^6$ cells) |
| **Release** | Cryopreserved drug product | Fresh drug product |
| **Time between apheresis and lymphodepletion** | Approximately 3 weeks | 9 days |
| **Time between apheresis and infusion** | Approximately 4 weeks | 14 days |
| **Safety switch** | Yes (inducible Caspase 9) | No |

Del Bufalo F, [….] Locatelli F. Blood 2023

# Expansion, Persistence and B-cell aplasia

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Del Bufalo F, [….] Locatelli F. Blood 2023 and update

# Toxicity of CD19-CAR_ALLO

| Toxicity | Number of patients (%) |
|---|---|
| CRS<br>- Grade 1-2<br>- Grade 3<br>- Grade 4 | 13/16 (81%)<br>13<br>0<br>0 |
| Neutropenia<br>- Grade 1-2<br>- Grade 3-4 | 16/16 (100%)<br>0<br>16 |
| Thrombocytopenia | 13/16 (81%) |
| Anemia | 15/16 (94%) |
| B-cell aplasia | 16/16 (100%) |
| ICANS | 1/16 (6%) |
| aGvHD | 2/16 (12%) |
| Capillary leak syndrome | 1/16 (6%) |

- 33 years old
- Treated with ALLO-CAR T cells 3 months after alloHSCT
- Previous gut a GvHD

- 27 years old
- Treated with ALLO-CAR T cells 4 months after alloHSCT
- Donor: MUD 9/10

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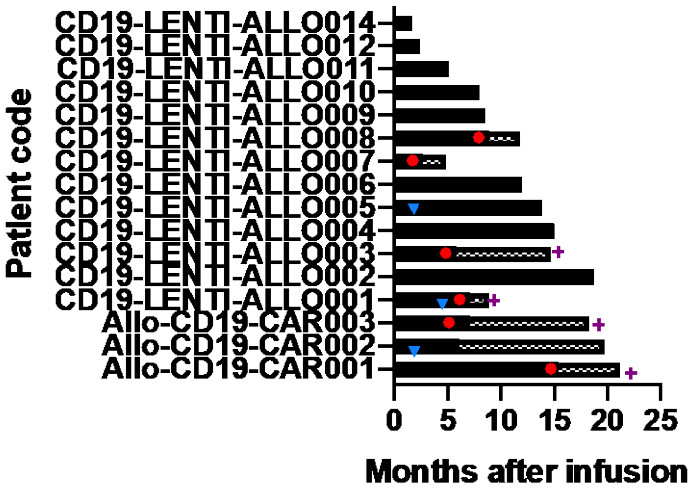
Del Bufalo F, [….] Locatelli F. Blood 2023 and update

# Disease course and long term outcomes





16 patients treated:
- 3 CD19-CAR-RETRO_ALLO
- 16 CD19-CAR-LENTI_ALLO

- 16/16 patients: MRD-negative CR in the BM at day +14
- 8/9 patients: CR at EM sites at day +28

3 patients received alloHSCT after ALLO-CAR T cells:
- 2 as first alloHSCT
- 1 as second alloHSCT

6 relapses:
- 4 isolated EM relapses
- 2 isolated BM relapse

10/16 patients maintain CR (median follow-up: 15 months, range 2-22)

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Data cut-off: 30th August 2023

# Conclusions

- Readily manufacturing of allogeneic CAR T cells is feasible
- Administration of ALLO_CAR-T cell is associated with a good tolerability profile and low incidence of aGVHD
- Upon infusion, ALLO_CAR-T cells expand, induce immune activation and show long term persistence
- ALLO_CAR-T cells have a strong antitumor activity that, in a significant proportion of patients, is maintained over time
- ALLO-CAR-T cell will be further evaluated in a prospective clinical trial
- ALLO-CAR-T cell may, in the future, be exploited to implement transplantation protocols in patients at higher risk of relapse

# ACKNOWLEDGEMENTS


## Department of Hematology/ Oncology

## Prof. Franco Locatelli

***Clinical Unit***

***Gene and Cell Therapy Unit***

***Concetta Quintarelli, Biagio De Angelis, Enrico Velardi, Mara Vinci***

Chiara Rosignoli

Marco Becilli

Giada Del Baldo

Matteo Amicucci

***Charité University Hospital - Berlin***

Annette Künkele, Arend von Stackelberg

**Cell Manipulation Unit**

Giuseppina Li Pira

Simone Biagini

Elia Girolami

**GMP Facility**

Monica Gunetti

Stefano Iacovelli

Rossana Bugianesi

Stefania Macchia

All Team!

**Flow cytometry**

Valentina Bertaina

Raffaella Sborgia

***All the patients and their families!***