3rd edition

Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

Turin, September 21-22, 2023

Starhotels Majestic

Scientific board:
Marco Ladetto (Alessandria)
Umberto Vitolo (Candiolo-TO)

CAR-T in adult ALL

Federico Lussana

Department of Oncology and Hematology, University of Milan
and Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII Bergamo

Disclosures of Federico Lussana

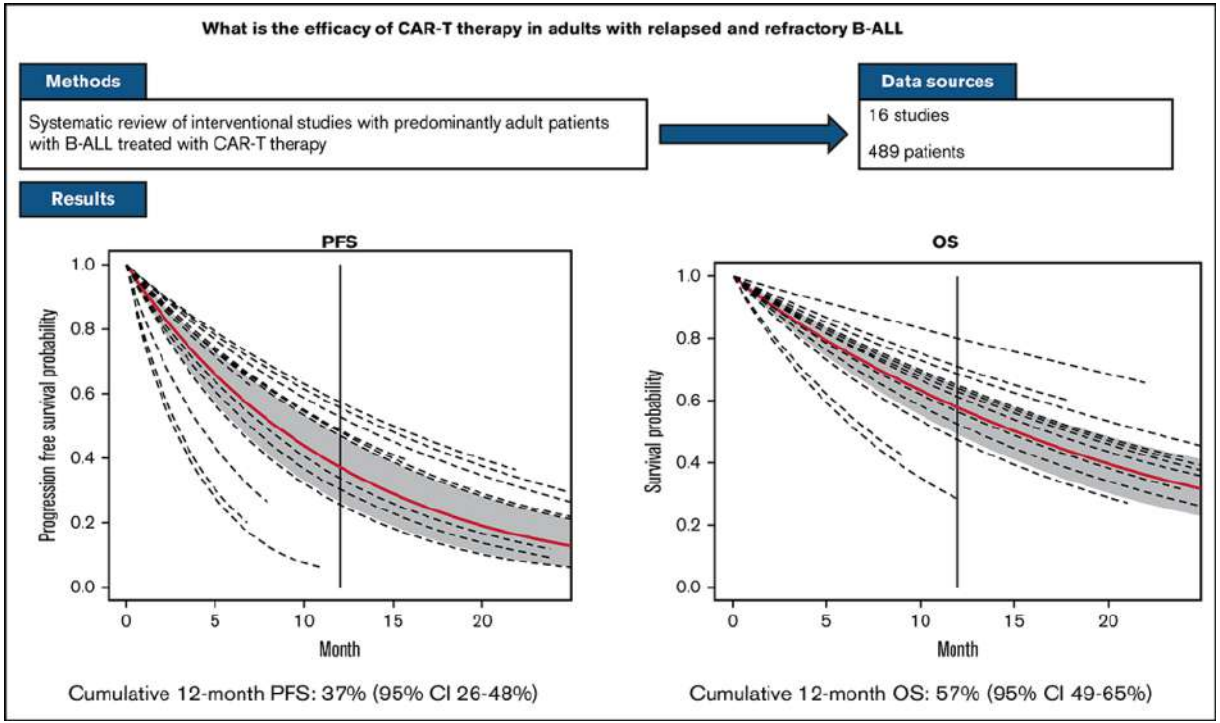
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Pfizer					X	X	
Abbvie					X	X	
Amgen					X		
Incyte					X		
Clinigen					X		
Bristol Myers Squibb					X	X	

Key anti-CD19 CAR-T cell therapy trials

	Eliana[1,2] (N=75)	CIBMTR[3] (N=249)	Zuma-3[4,5] (N=55)	US[6] (N=76)
CAR-T cell agent	**Tisagenleucel**	**Tisagenleucel**	**Brexucabtagene**	**Brexucabtagene**
Study phase	II	II	II	II
Co-Stimolatory domain	4-1BB	4-1BB	CD28	CD28
Study population	Pediatric/young adults (<26y)	Pediatric/young adults (<26y)	Adults R/R B-ALL	Adults R/R B-ALL
CR, %	MRD negative 81	85	Overall 71	MRD negative 83
OS, %	55 (at 5 years)	77 (at 1 year)	59 (at 2 years)	87 (at 6-month)
EFS, %	42 (at 5 years)	52 (at 1 year)	40 (at 2 years)	59 (at 6-month)
	FDA Approved 2017		**FDA Approved 2021**	

1 Maude et al. NEJM 2018; 2 Rives et al. EHA HemaSphere 2022; 3 Pasquini MC, et al. Blood Advances 2020; 4 Shah et al. Lancet 2021; 5 Shah et al. J Clin Oncol 40:7010, 2022; Roloff G et al. JCO 2023

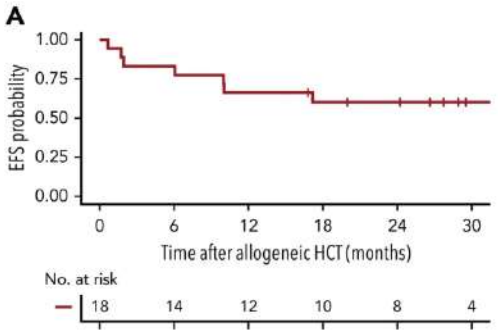
CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN ADULTS WITH B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA



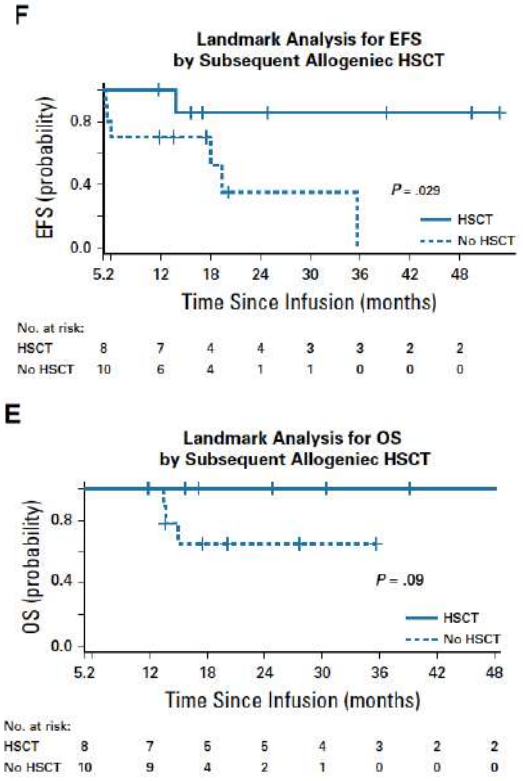
Punita Grover et al. Blood Adv, 2022

CAR-T in ALL
only a bridge to alloHSCT?

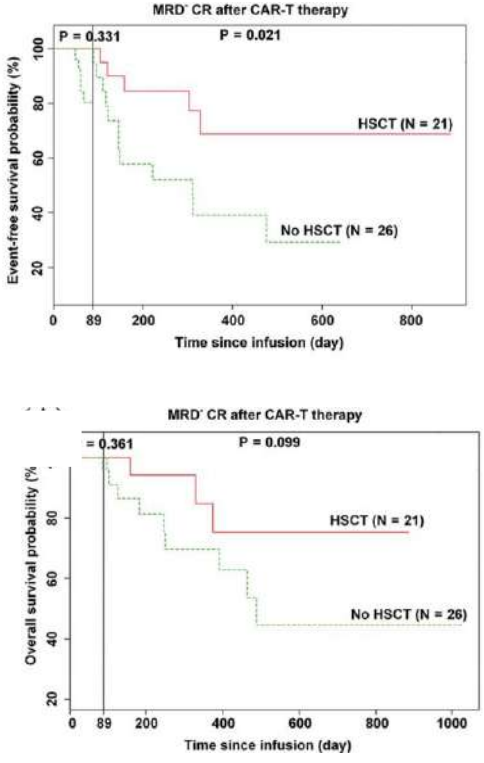
HCT may improve EFS following CD19 CAR in some published studies



Hay, et al. Blood 2019

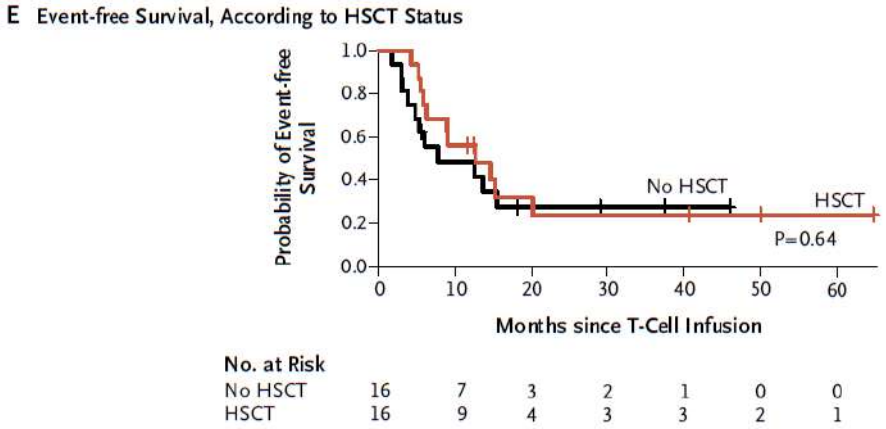


Frey, et al. JCO 2020



Jiang, et al. AJH 2019

Long-Term outcomes with CAR-T cells in adults with R/R B-ALL



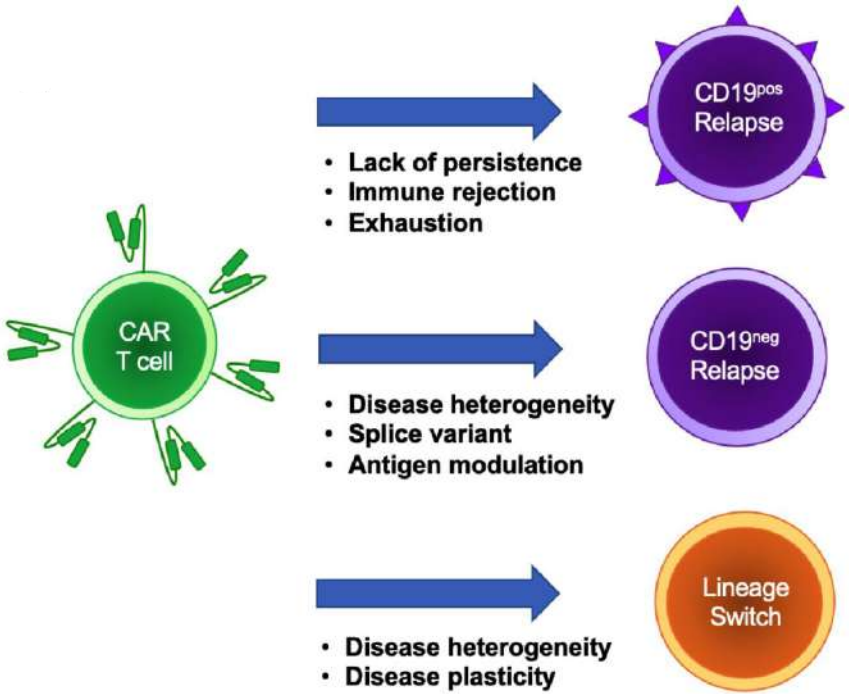
JH Park et al. N Engl J Med 2018;378:449-459.

- Role of HCT after CAR-T is controversial, especially in adults
- In adults patients the risk/benefit of alloHSCT is less clear given the increased risk of non-relapse death following transplants
- Neither trial demonstrated an obvious survival benefit to alloHSCT following CAR T cell therapy

How to best predict long-term response after CAR-T?

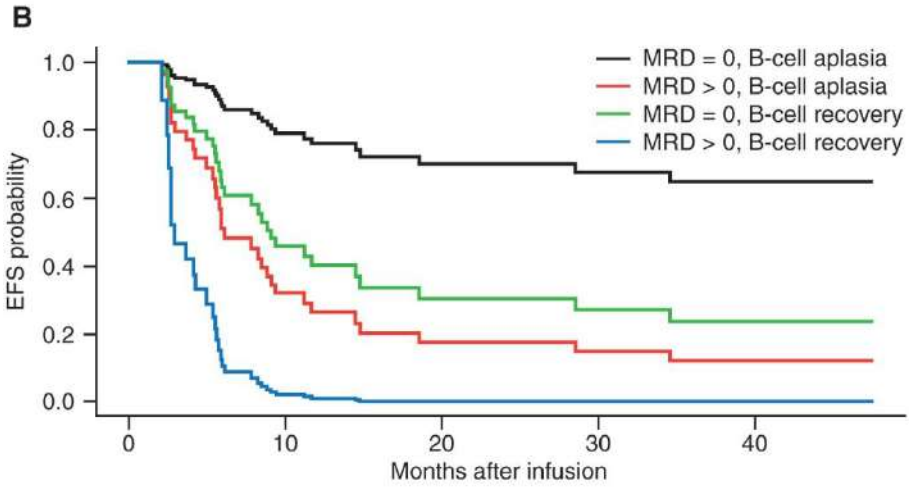
Pre-infusion factors impacting relapse immunophenotype following CD19 CAR T cells

- High pre-infusion TB
- Non-CNS active extramedullary disease
- Blinatumomab non-response
- CD19/ 28ζ CAR construct
- KMT2A rearrangement



Lamble AJ et al.: , Blood Advances2022; Myers RM et al, Blood 2023; Shah NN et al. J Clin Oncol 2021; Schultz LM, et al. J Clin Oncol. 2022 ; Kadauke S J Clin Oncol 2021

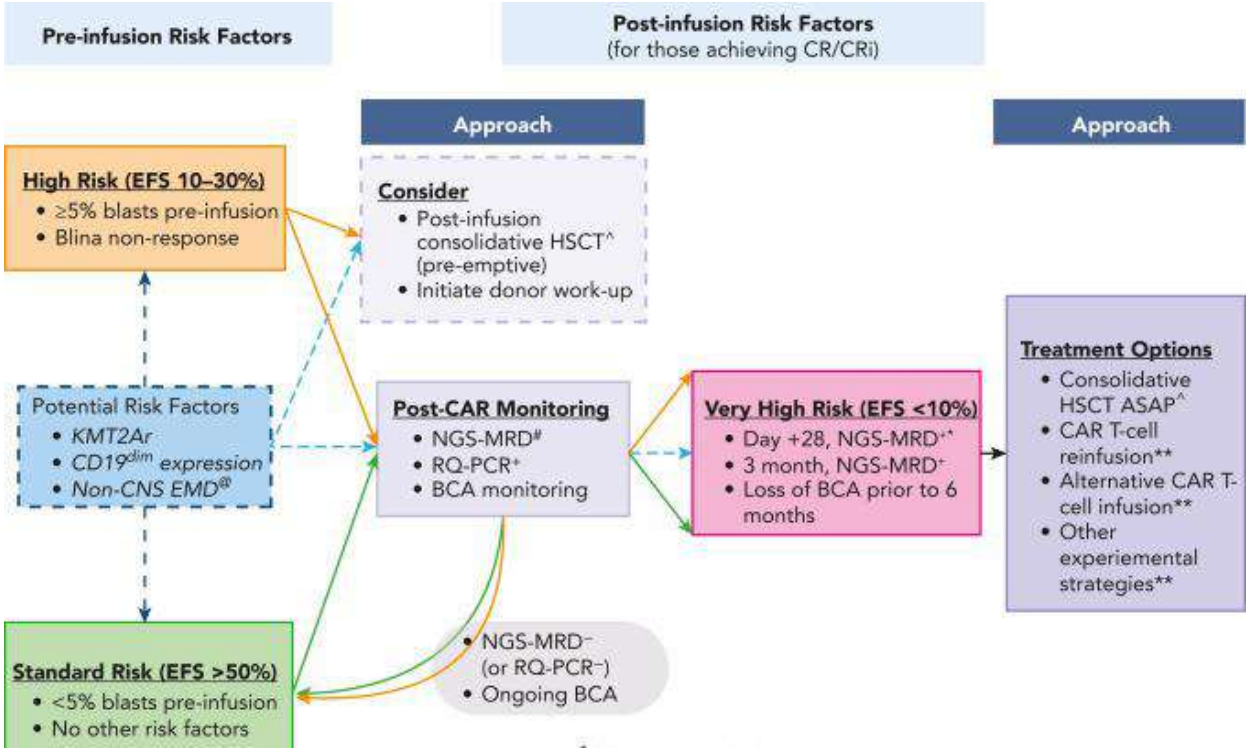
Combined model of B cell aplasia >1 year and NGS MRD



- BM-NGS MRD is the best biomarker for risk of relapse at any time throughout the first year
- B cell aplasia during the first year is also a strong biomarker
- Pts who lose B-cell aplasia <6 months or develop NGS-MRD>0 on BM are at high risk of relapse

Pulsipher et al. Blood Cancer Discovery 2022

How to approach alloHSCT after CAR-T



…however, no trial was designed to answer the question whether transplantation after CAR-T is needed

Myers et al. Blood 2023

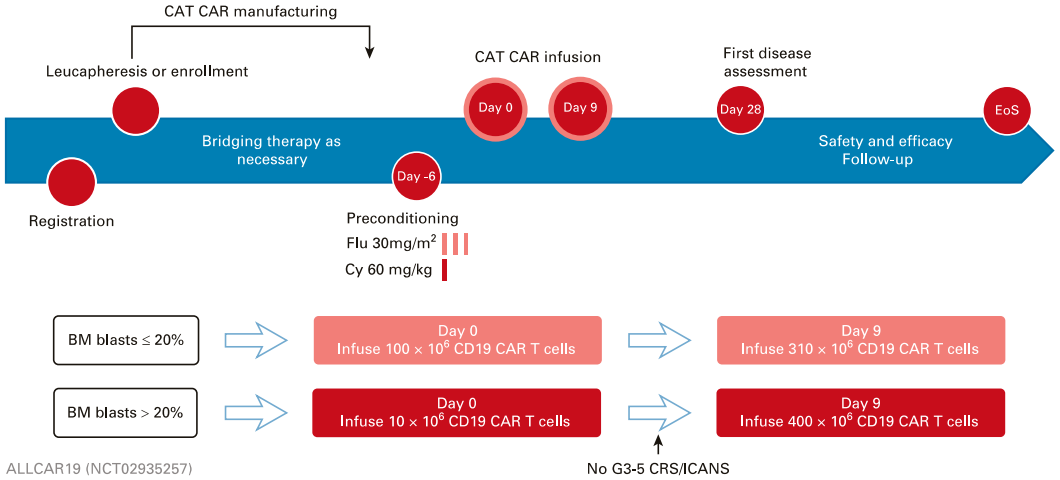
CAR-T: will be next gen more durable?

Ongoing trials are evaluating:

- novel CD19 constructs
- allogeneic off-the shelf CAR-T therapies

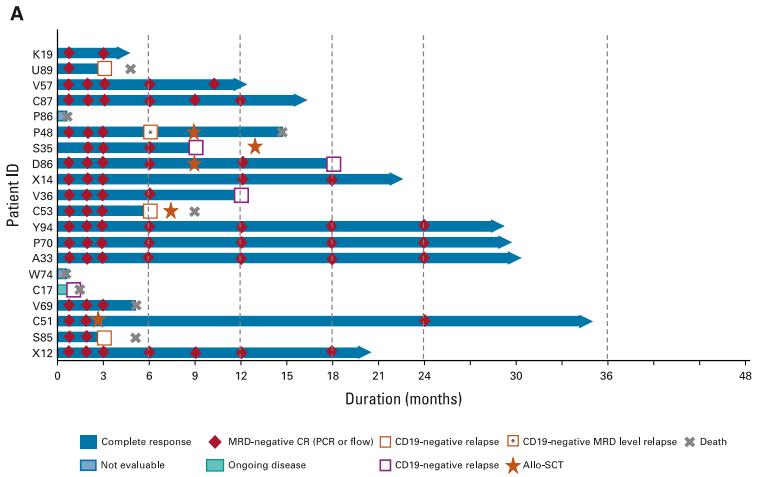
Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell ALL

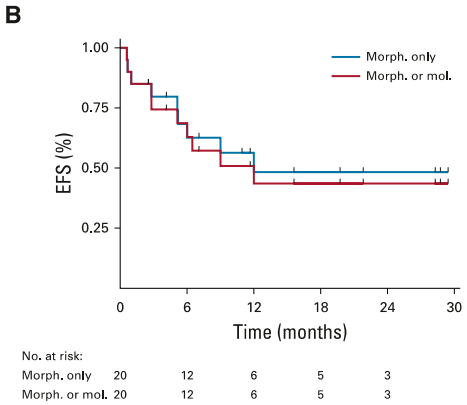
Baseline Characteristic	n = 20
Sex, No. (%)	
Female	7 (35)
Male	13 (65)
Median age, years (range)	41.5 (18-62)
Chromosomal or molecular status, No. (%)	
Ph+ (bcr-abl)	6 (30)
MLL	1 (5)
Others	8 (40)
Normal	4 (20)
Failed	1 (5)
Previous treatment	
Median previous lines (range)	3 (2-6)
Inotuzumab ozogamicin exposure, No. (%)	10 (50)
Blinatumomab exposure, No. (%)	5 (25)
Previous allo-SCT, No. (%)	13 (65)

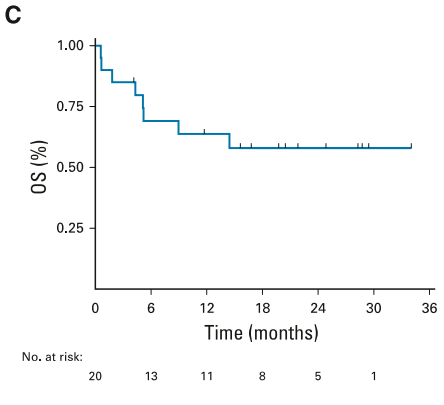


Roddie C et al.: Journal of Clinical Oncology 39:3352-3363. 2021

Durable Responses and Low Toxicity After Fast Off-Rate CD19 Chimeric Antigen Receptor-T Therapy in Adults With Relapsed or Refractory B-Cell ALL



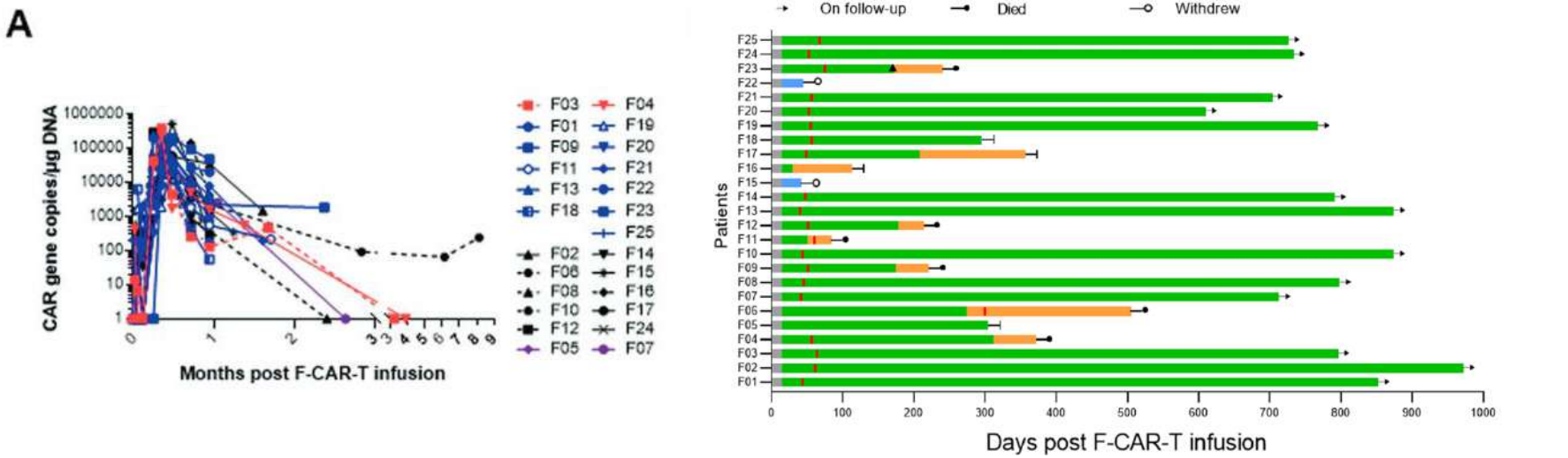




Roddie C et al.: Journal of Clinical Oncology 39:3352-3363. 2021

Next-day manufacture of a novel anti-CD19 CAR-T therapy for B-ALL: first-in-human clinical study

Clinical outcome

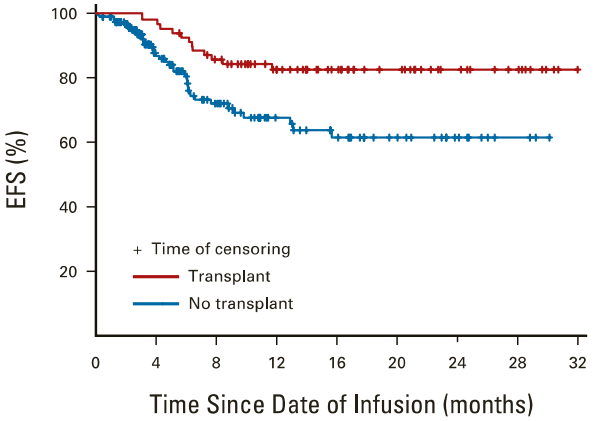


Yang J et al.: Blood Cancer Journal (2022) 12:104 ; https://doi.org/10.1038/s41408-022-00694-6

Co-administration of CD19- and CD22-Directed CAR-T Cell Therapy in Childhood B-Cell ALL: A Single-Arm, Multicenter, Phase II Trial

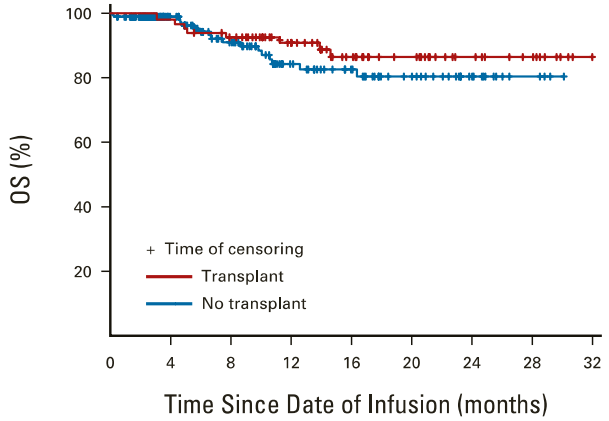
Results

- Patients registered (N = 232); infused (N= 225); achieving CR (N= 192);
- Patients consolidated with transplant (N= 78) (due to KMT2A rearrangement, (n = 22), ZNF384 fusion (n = 2), parent request (n = 54)



No. at risk (No. censored):

Transplant	78 (0)	77 (0)	66 (2)	50 (16)	34 (32)	24 (42)	15 (51)	10 (56)	1 (65)
No transplant*	194 (0)	104 (72)	64 (97)	39 (119)	29 (126)	18 (137)	11 (144)	4 (151)	0 (155)

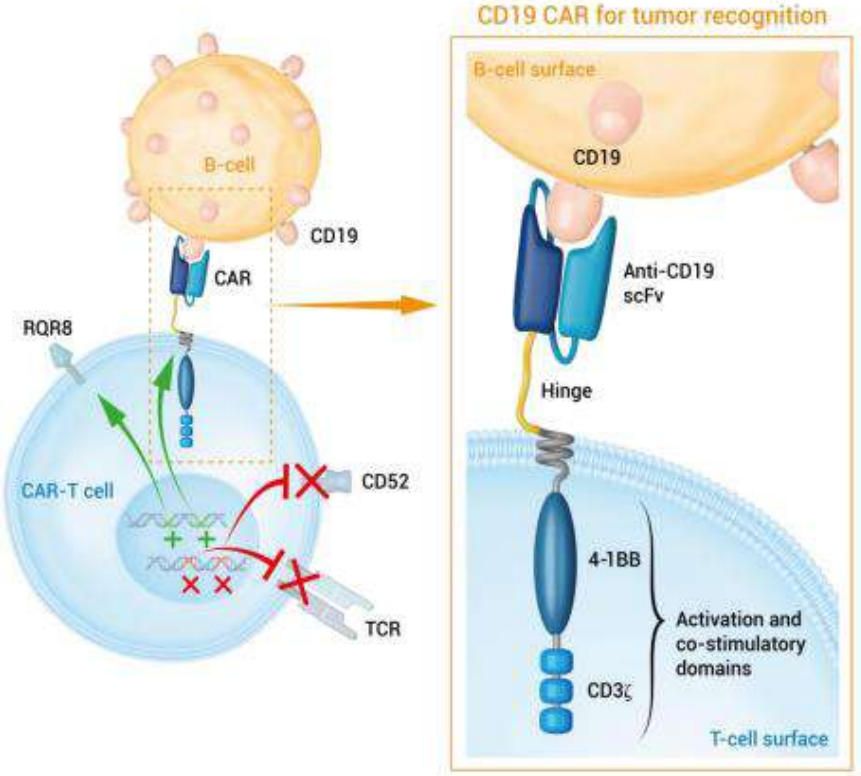


No. at risk (No. censored):

Transplant	78 (0)	77 (0)	71 (2)	54 (18)	35 (35)	25 (45)	15 (55)	10 (60)	1 (69)
No transplant*	194 (0)	120 (72)	86 (98)	55 (124)	42 (136)	26 (151)	15 (162)	5 (172)	0 (177)

Wang T et al.: JCO 2022 DOI https://doi. org/10.1200/JCO.22. 01214

Off-the-shelf genome-editing allogeneic anti-CD19 CAR-T: UCART



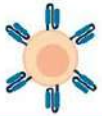
- 25 patients of median age 37 years (IQR 28–45)
- Two (8%) patients developed grade 1 acute cutaneous graft-versus-host disease
- CR/CRi rate: 12 out of 25 (48%)
- After a median of follow-up of 12.8 months, overall response rate was 48%
- Median PFS was 2.1 months and OS was 13.4 months

R. Benjamin, Lancet Hematol 2022

Allogeneic, Donor-Derived, Second-Generation, CD19-CAR-T Cells for the Treatment of Pediatric Relapsed/Refractory B-Cell-Precursor Acute Lymphoblastic Leukemia (BCP-ALL)

Background. Autologous CD19.CAR-T cells have non-negligible limitations, including:

- Manufacturing issues (particularly in patients with profound lymphopenia);
- Potential blast contamination of the apheretic product;
- Limited persistence after infusion.



Patients and Methods

13 children/young adults received allogeneic donor-derived 2nd generation (4.1BB) CD19.CAR T cells generated using 2 different constructs and manufacturing processes

CD19-CAR-Retro_ALLO:
- Retroviral construct including the suicide gene inducible caspase-9;
- Manual manufacturing;
- Cryopreserved, bulky apheresis as starting material;
- Cryopreserved final product.

CD19-CAR-Lenti_ALLO:
- Lentiviral construct;
- Automated, Prodigy®-based, manufacturing process;
- Fresh apheresis and $CD4^+/CD8^+$ selected T cells as starting material;
- Fresh final product.

Outcomes

13 patients treated:
- 3 CD19-CAR-Retro_ALLO
- 10 CD19-CAR-Lenti_ALLO

- 13/13 patients: MRD-negative CR in the BM at day +14
- 5/6 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

5 relapses:
- 4 isolated EM relapses
- 1 isolated BM relapse

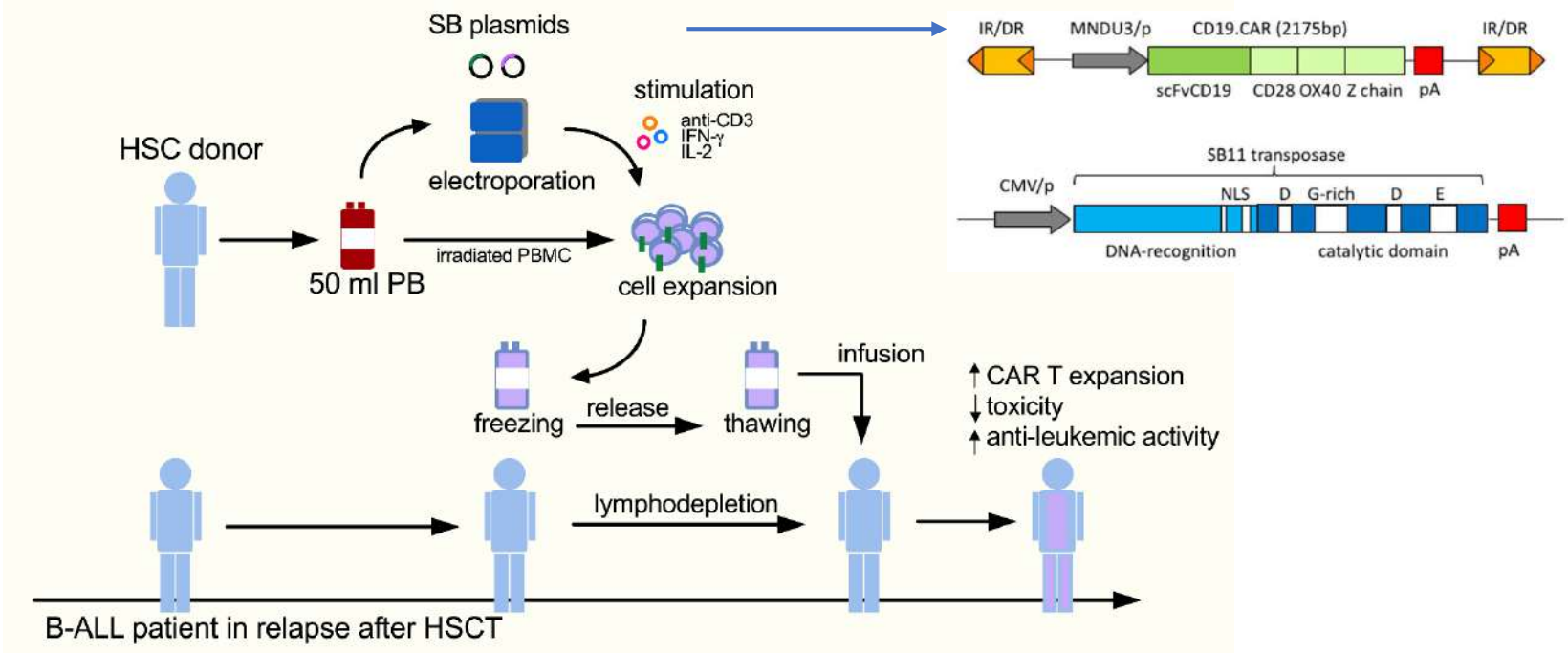
8/13 patients maintain CR (median follow-up: 12 months, range 5-21)

Conclusion: Allogeneic anti-CD19 CAR-T cells can be successfully generated to effectively treat refractory BCP-ALL relapsing after allogeneic stem cell transplantation, without increased toxicity as compared to autologous CAR-T cells.

Del Bufalo et al. Blood 2023

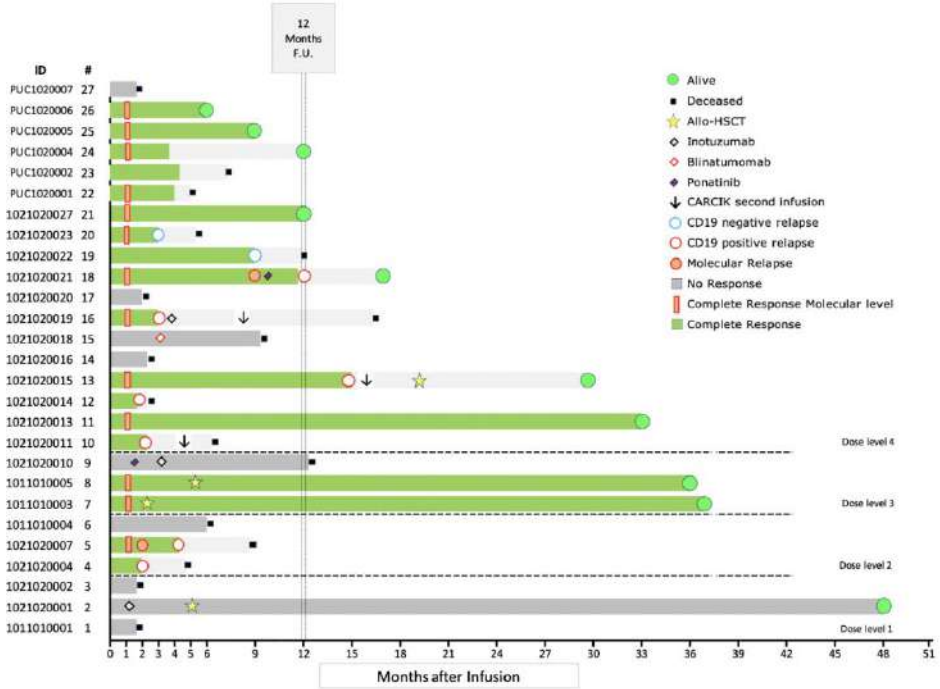
Phase I/IIa trial with SB-engineered CARCIK-CD19 in B-ALL post HSCT

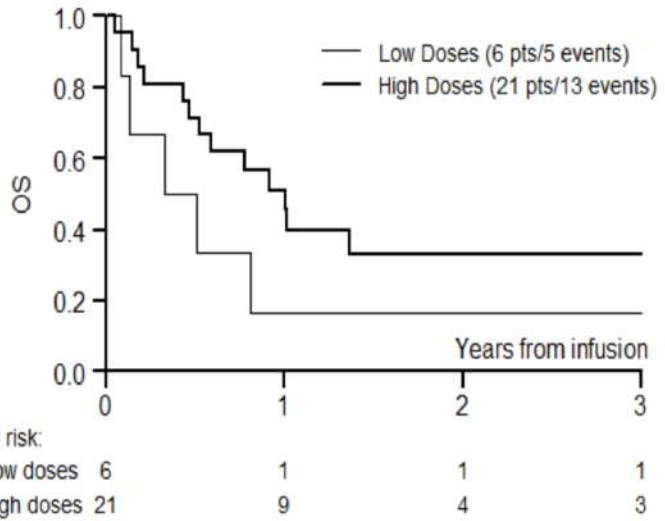
Multicenter Dose Escalation Study FT01CARCIK (NCT03389035) and compassionate use FT02CARCIK
Enrolling in ASST-Monza and ASST-Bergamo, IT
Manufactured in-house



Magnani CF, J Clin Invest. 2020 Nov 2;130(11):6021-6033

Response data





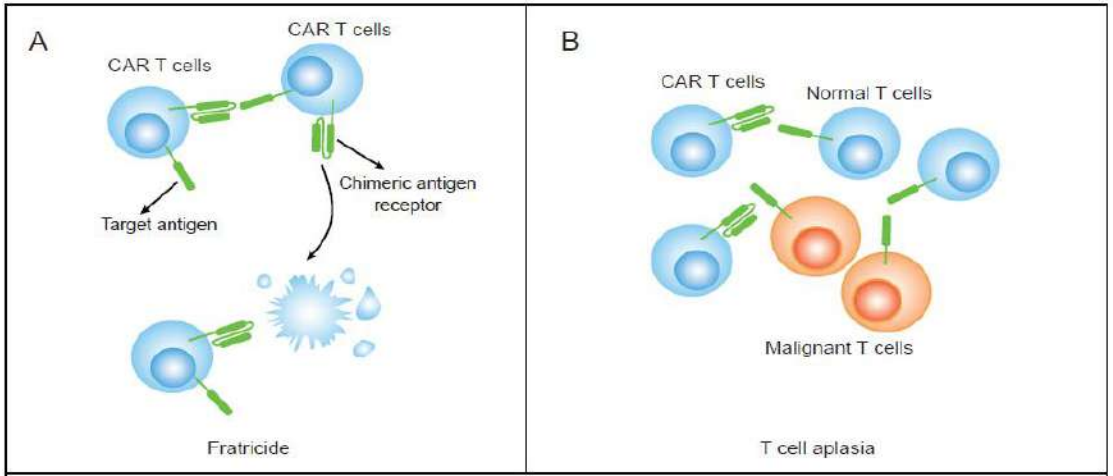
- Secondary GvHD was not observed
- CR: 77% of patients treated with the 2 highest doses (MRD neg 81%)

Lussana F. et al. ASH 2022

Arrival of CAR-T for T-ALL

Thee major challenges for CAR-T cell therapy in T-ALL

A. Fratricide



B. T-cell aplasia

C. Product contamination

Ren, A.; Tong, X.; Xu, N.; Zhang, T.; Zhou, F.; Zhu, H. Vaccines 2023

Donor-Derived CD7 Chimeric Antigen Receptor T Cells for T-Cell Acute Lymphoblastic Leukemia: First-in-Human, Phase I Trial

Design

- To minimize CD7 CAR T-cell–mediated fratricide, a CD7-targeting CAR construct using IntraBlock technology, which prevents CD7 cell surface expression
- Anti-CD7 CAR T cells, manufactured from either previous stem-cell transplantation donors or new donors, to patients with r/r T-ALL
- Single infusions at doses of 5×10^5 or 1×10^6 (±30%) cells per kilogram of body weight
- The primary end point was safety with efficacy secondary

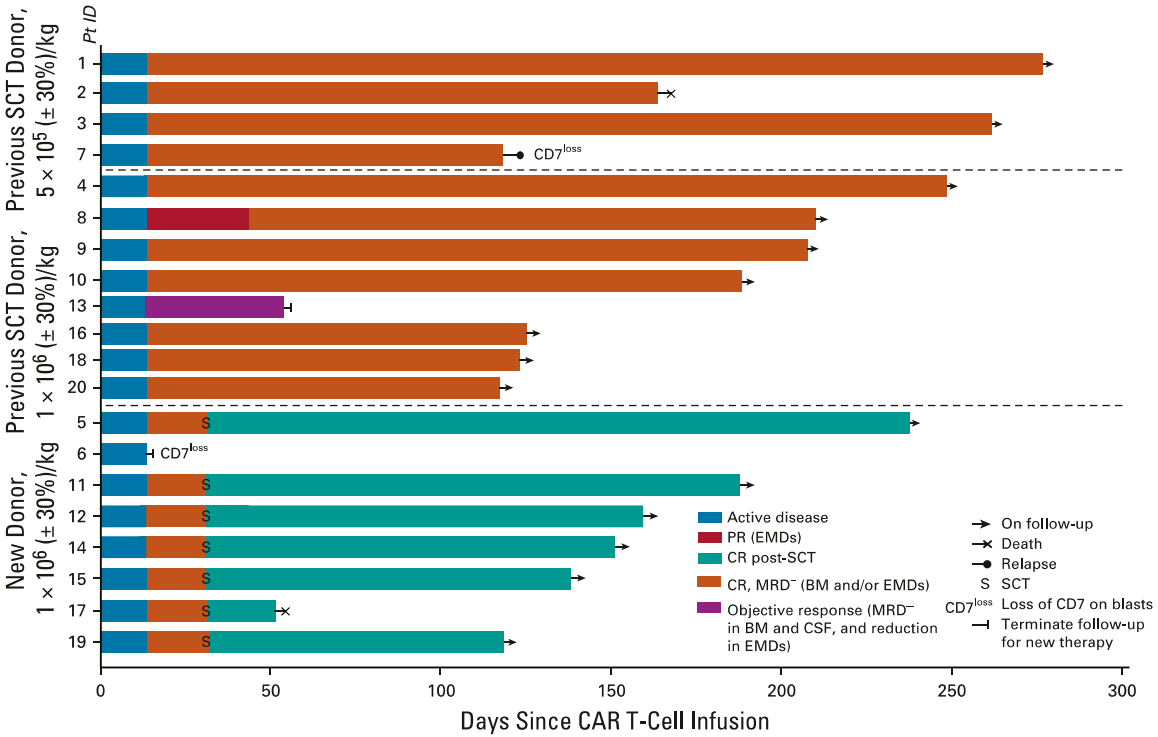
Safety

AE	Grade 1	Grade 2	Grade 3	Grade 4
CRS				
Total score	10 (50)	8 (40)	1 (5)	1 (5)
Fever	20 (100)	0	0	0
Hypoxia	0	8 (40)	1 (5)	1 (5)
Hypotension	0	0	2 (10)	0
ICANS				
Total score	3 (15)	0	0	0
ICE score	3 (15)	0	0	0
Depressed consciousness	0	0	0	0
Seizure	0	0	0	0
Motor weakness	0	0	0	0
Elevated ICP or cerebral edema	0	0	0	0
GVHD				
Total score	11 (55)	1 (5)	0	0
Skin	12 (60)	0	0	0
Liver	0	1 (5)	0	0
Intestinal	0	0	0	0

Pang J et al.: Journal of Clinical Oncology 39, no. 30 (October 20, 2021) 3340-3351

Donor-Derived CD7 Chimeric Antigen Receptor T Cells for T-Cell Acute Lymphoblastic Leukemia: First-in-Human, Phase I Trial

- Ninety percent achieved CR, including 85% (n = 17) who achieved MRD-negative CR by day 15
- At a median follow-up of 6.3 months, 75% of patients remained in remission
- CAR T cells were still detectable in five of five patients assessed in month 6, postinfusion.



Pang J et al.: Journal of Clinical Oncology 39, no. 30 (October 20, 2021) 3340-3351

Base-Edited CAR7 T Cells

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia

Robert Chiesa, M.D., Christos Georgiadis, Ph.D., Farhatullah Syed, Ph.D., Hong Zhan, Ph.D., Annie Etuk, Ph.D., Soragia Athina Gkazi, Ph.D., Roland Preece, Ph.D., Giorgio Ottaviano, M.D., Toni Braybrook, M.Bio., Jan Chu, M.Sc., Agnieszka Kubat, B.Sc., Stuart Adams, Ph.D., Rebecca Thomas, Ph.D., Kimberly Gilmour, Ph.D., David O'Connor, M.B., Ch.B., Ajay Vora, M.B., B.S., and Waseem Qasim, M.B., B.S., Ph.D., for the Base-Edited CAR T Group*

ABSTRACT

BACKGROUND

Cytidine deamination that is guided by clustered regularly interspaced short palindromic repeats (CRISPR) can mediate a highly precise conversion of one nucleotide into another — specifically, cytosine to thymine — without generating breaks in DNA. Thus, genes can be base-edited and rendered inactive without inducing translocations and other chromosomal aberrations. The use of this technique in patients with relapsed childhood T-cell leukemia is being investigated.

METHODS

We used base editing to generate universal, off-the-shelf chimeric antigen receptor (CAR) T cells. Healthy volunteer donor T cells were transduced with the use of a lentivirus to express a CAR with specificity for CD7 (CAR7), a protein that is expressed in T-cell acute lymphoblastic leukemia (ALL). We then used base editing to inactivate three genes encoding CD52 and CD7 receptors and the β chain of the $\alpha\beta$ T-cell receptor to evade lymphodepleting serotherapy, CAR7 T-cell fratricide, and graft-versus-host disease, respectively. We investigated the safety of these edited cells in three children with relapsed leukemia.

RESULTS

The first patient, a 13-year-old girl who had relapsed T-cell ALL after allogeneic stem-cell transplantation, had molecular remission within 28 days after infusion of a single dose of base-edited CAR7 (BE-CAR7). She then received a reduced-intensity (nonmyeloablative) allogeneic stem-cell transplant from her original donor, with successful immunologic reconstitution and ongoing leukemic remission. BE-CAR7 cells from the same bank showed potent activity in two other patients, and although fatal fungal complications developed in one patient, the other patient underwent allogeneic stem-cell transplantation while in remission. Serious adverse events included cytokine release syndrome, multilineage cytopenia, and opportunistic infections.

CONCLUSIONS

The interim results of this phase 1 study support further investigation of base-edited T cells for patients with relapsed leukemia and indicate the anticipated risks of immunotherapy-related complications. (Funded by the Medical Research Council and others; ISRCTN number, ISRCTN15323014.)

From Great Ormond Street Hospital for Children NHS Trust (R.C., C.O., T.B., J.C., S.A., R.T., K.G., D.O., A.V., W.Q.) and the UCL Great Ormond Street Institute of Child Health (C.G., F.S., H.Z., A.E., S.A.G., R.P., A.K., W.Q.) — both in London. Dr. Qasim can be contacted at w.qasim@ucl.ac.uk or at Great Ormond Street Hospital, 20 Guilford St., London, WC1N 1DZ, United Kingdom.

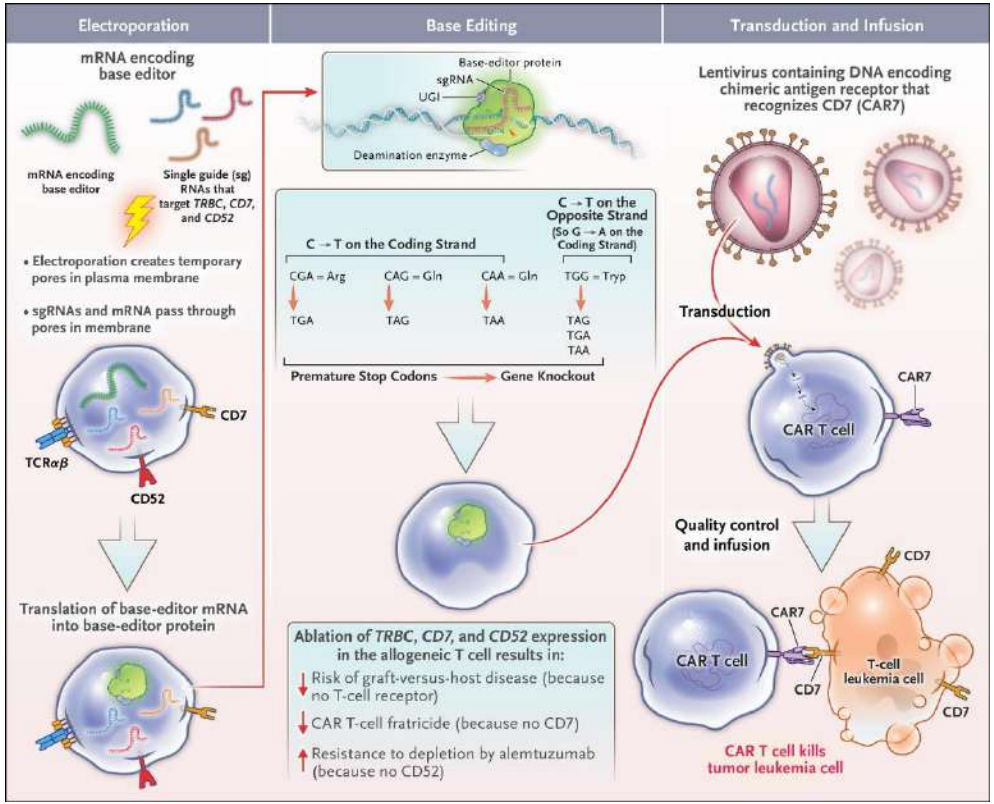
*A list of members of the Base-Edited CAR T Group is provided in the Supplementary Appendix, available at NEJM.org.

Drs. Chiesa and Georgiadis contributed equally to this article.

This article was published on June 14, 2023, at NEJM.org.

This is the *New England Journal of Medicine* version of record, which includes all Journal editing and enhancements. The Author Accepted Manuscript, which is the author's version after external peer review and before publication in the Journal, is available at PubMed Central.

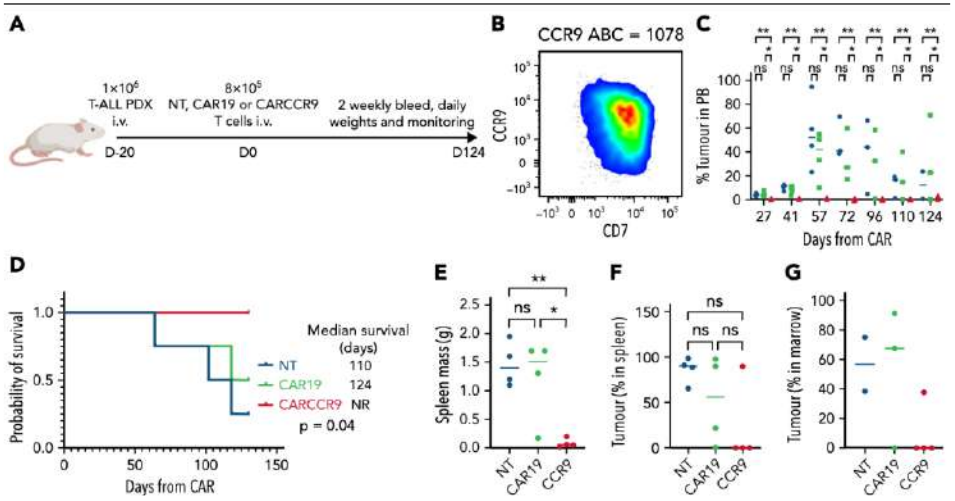
N Engl J Med 2023;388:899-910.
DOI: 10.1056/NEJMoa2300709



Chiesa et al. NEJM June 2023

Anti-CCR9 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia

- The chemokine receptor CCR9 is expressed in >70% of cases of T-ALL, including >85% of relapsed/refractory disease, and only on a small fraction (<5%) of normal T cells
- CAR-T cells targeting CCR9 are resistant to fratricide and have potent antileukemic activity both in vitro and in vivo
- anti-CCR9 CAR-T cells could be a highly effective treatment strategy for T-ALL, avoiding T cell aplasia and the need for genome engineering that complicate other approaches



PM Maciocia et al: Blood 2022 Jul 7;140(1):25-37.

Conclusions

- CAR T cell therapy induces MRD-negative remissions for many patients with relapsed or refractory B-ALL
- Factors such as patient TB, active extramedullary disease, post-infusion NGS-MRD, presence of BCA, should all inform the decision on consolidative alloHSCT following CAR T cell therapy for B-ALL
- Rapid progress is ongoing with new generation of CAR-T cells using different cell platforms and allogeneic donors
- Preclinical data and early phase trial data are demonstrating impressive efficacy with multiple different approaches for T-ALL

Acknowledgments

Hematology and Bone Marrow Transplant Unit, ASST Papa Giovanni XXIII, BG and Università degli Studi di Milano, Milan, Italy

Alessandro Rambaldi
Giuseppe Gritti
Silvia Ferrari
Anna Grassi
Benedetta Rambaldi
Giuliana Rizzuto
Gian Maria Borleri
Cristian Meli

The Cell Therapy Lab, Gilberto Lanzani ASST Papa Giovanni XXIII, BG, Italy

Martino Introna
Chiara Capelli
Elisa Gotti
Josee Golay
Silvia Zaninelli

Cell Factory- Laboratorio di Terapia Cellulare e Genica Stefano Verri, ASST-Monza, Ospedale San Gerardo, Monza, Italy

Chiara Magnani
Giuseppe Gaipa
Daniela Belotti
Giada Matera
Benedetta Cabiati
Stefania Cesana
Valentina Colombo
Michele Quaroni

Tettamanti Research Center, Department of Pediatrics, University of Milano-Bicocca, Monza, Italy

Andrea Biondi
Giuseppe Dastoli
Ettore Biagi
Sarah Tettamanti
Chiara Buracchi
Silvia Rigamonti
Grazia Fazio
Giovanni Cazzaniga