Penn Medicine

Gene Editing: The Next Frontier for CAR T

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T. HOWARD LEE KEYNOTE LECTURE
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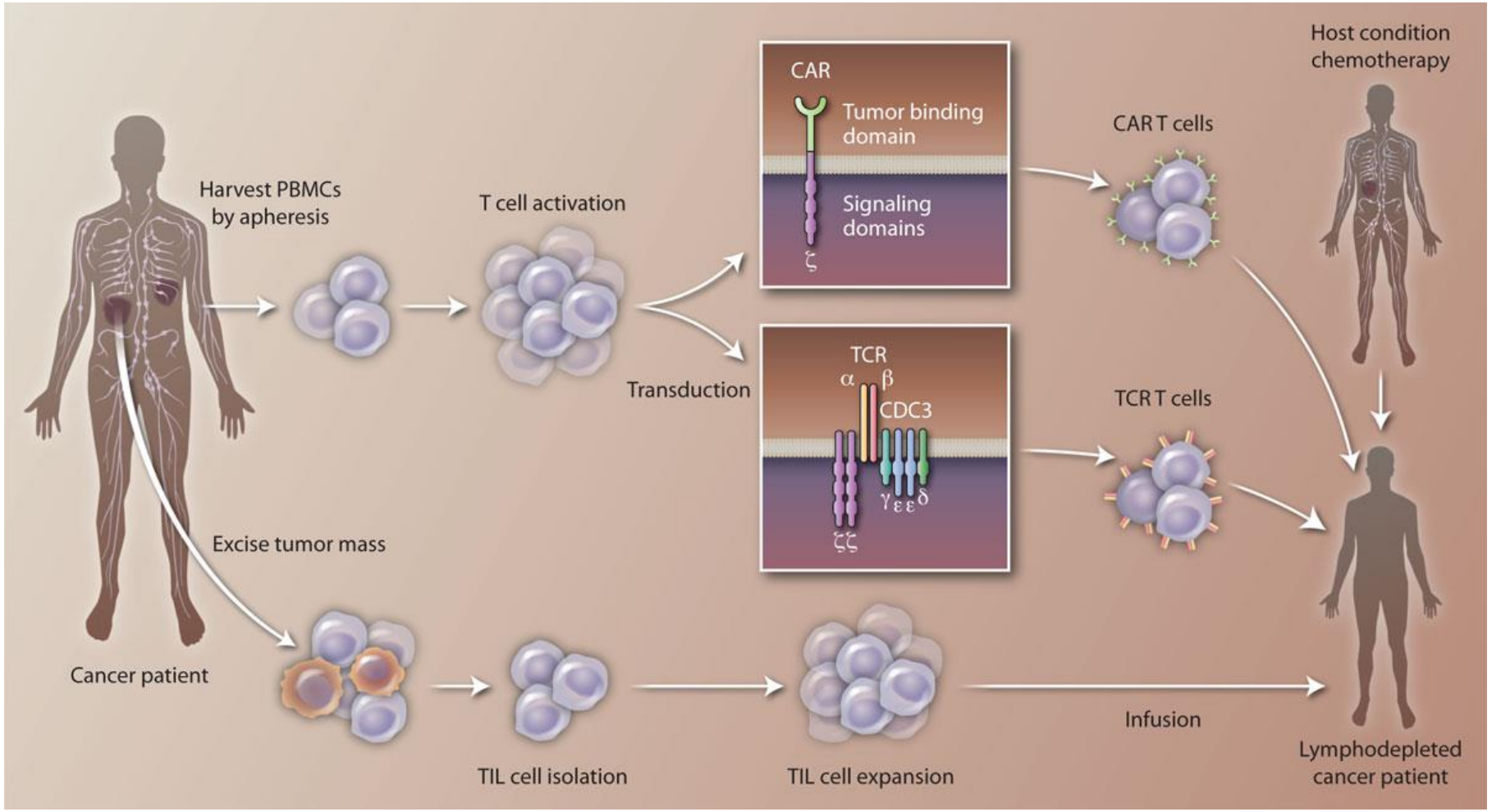
the cure is within
ABRAMSON CANCER CENTER

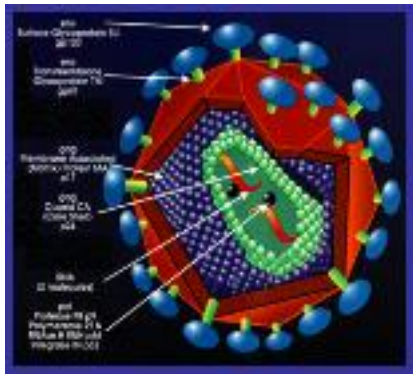
Adoptive T cell therapy (three major approaches)



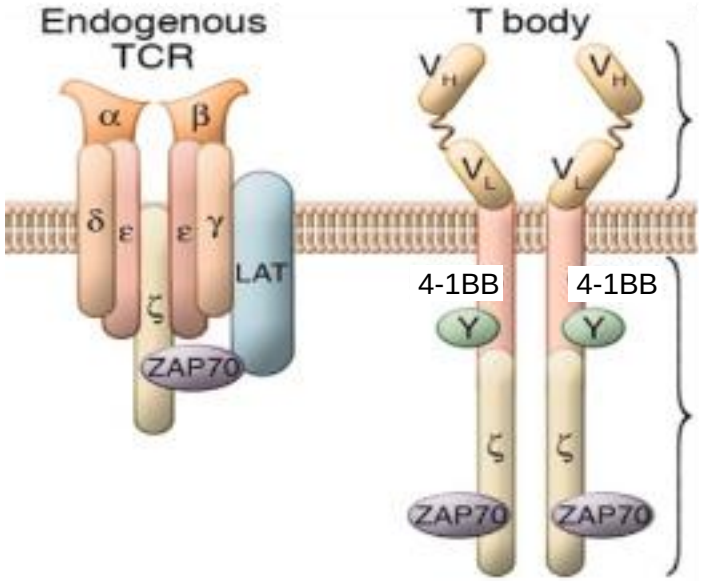
June et al *Sci Trans Med 2015*

CAR for B Cell Malignancy:

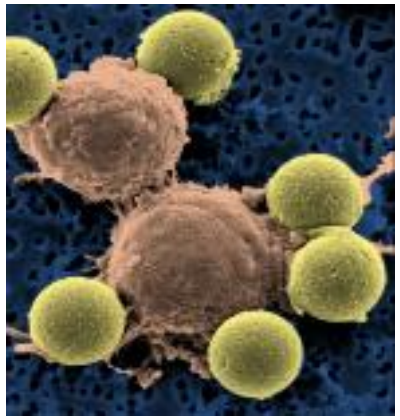
Autologous T Cells Transduced w/ Anti-CD19 Receptor Spliced to CD3 zeta and 4-1BB Signaling Domains



Lentiviral vector to deliver construct



CD3-z and 4-1BB signaling domains augments proliferation and survival



Anti-CD3/anti-CD28 mab coated bead stimulation (artificial DC) Expands the cells

Adapted from: Maus MV, et al. Blood. 2014;123:2625-35.

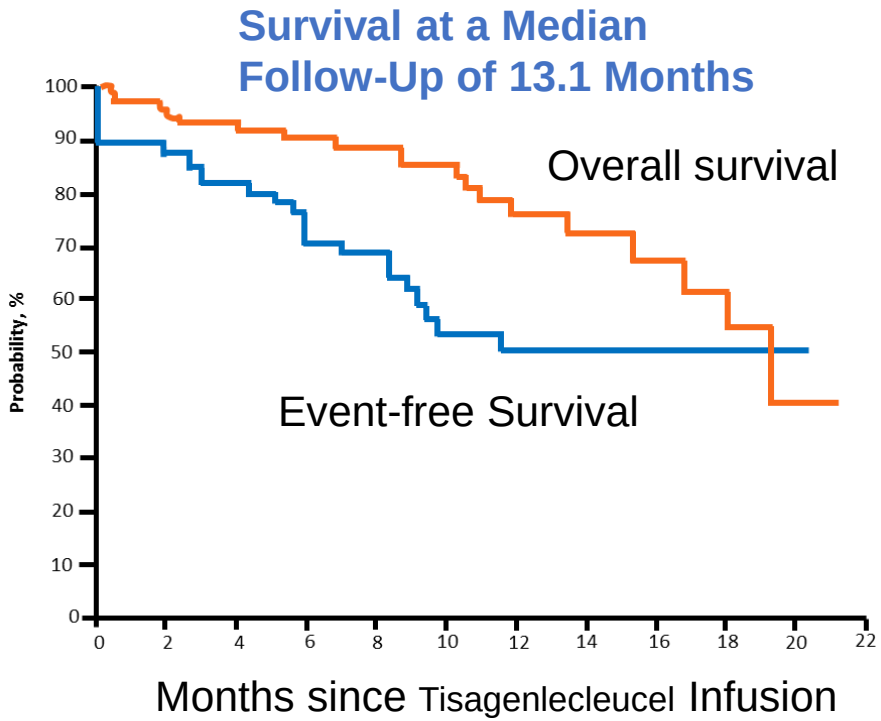
Successes of CART19 Therapy

Ref	Program/ CAR	Population	Response
Acute Lymphoblastic Leukemia			
Maude et al. NEJM 2014	PENN 4-1BB	N=30(ALL) Peds&Adults	CR=90%
Davila et al. SciTrMed 2014	MSK CD28	N=16 (ALL) Adults	CR=88%
Lee et al. Lancet 2015	NCI CD28	N=21 (ALL) Peds&AYA	CR=67% Intent to Treat
Turtle et al. JCI 2016	Seattle 4-1BB	N=30 Adults	CR=93%
Non-Hodgkins Lymphoma & Chronic Lymphocytic Leukemia			
Kochenderfer JCO 2015	NCI CD28	N=15 (NHL/CLL)	CR=53% PR=27%
Porter et al. SciTrMed2014	PENN 4-1BB	N=14(CLL)	CR=29% PR=29%

ELIANA: CAR T-cell Therapy in ALL

- Phase II trial of CAR T-cell therapy: **tisagenlecleucel**
- 79 pediatric/young adult patients (age 3-23) with relapsed or refractory CD19+ B-cell acute lymphoblastic leukemia (ALL)
- Median duration of remission and median overall survival remain unreached

24 month follow up analysis →

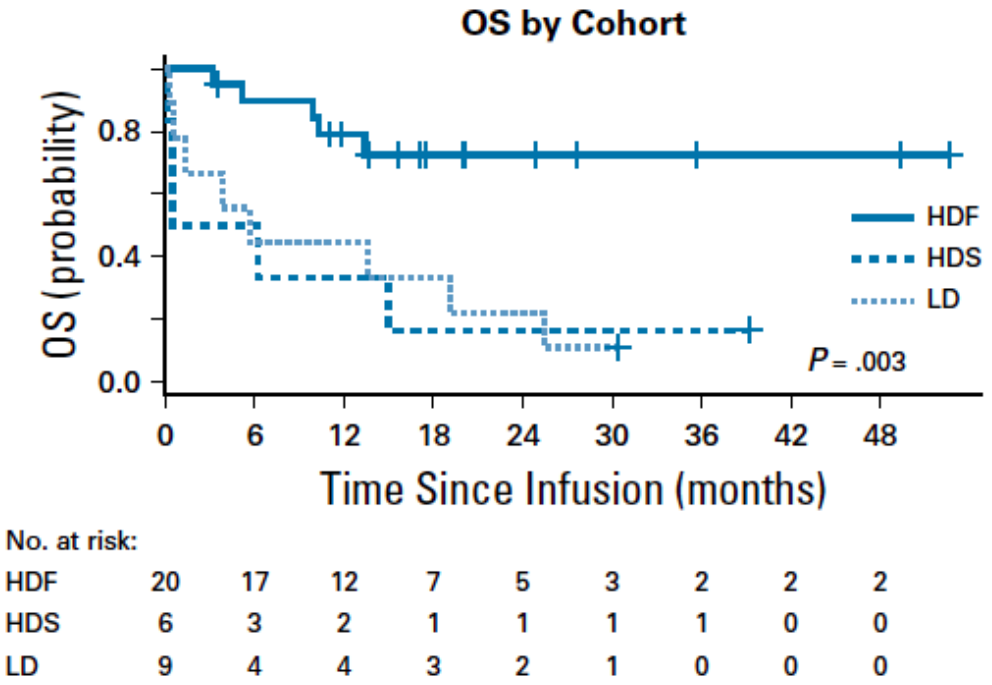


	Event Free Survival	Overall Survival
12 months	66%	76%
18 months	66%	70%
24 months	62%	66%

Grupp SA, et al. Presented at 60th American Society of Hematology Annual Meeting; December 1-4, 2018; San Diego, CA. Abstract 895. Maude SL, et al. N Engl J Med. 2018;378:439-448.

Adults wih ALL treated with CART-19

- Demographics:
  - Median age 34 YO (range 20-70)
  - Median of 3 prior regimens (blina/HSCT in >30%)
- 3 cohorts
  - Low dose (LD)
  - High dose, single (HDS)
  - High dose, fractionated (HDF)
- CR rate 69%
  - HDF 90% (18/20)
  - LD + HD = 40% (6/15)
  - All CRs MRD(-) by flow (<0.01%)

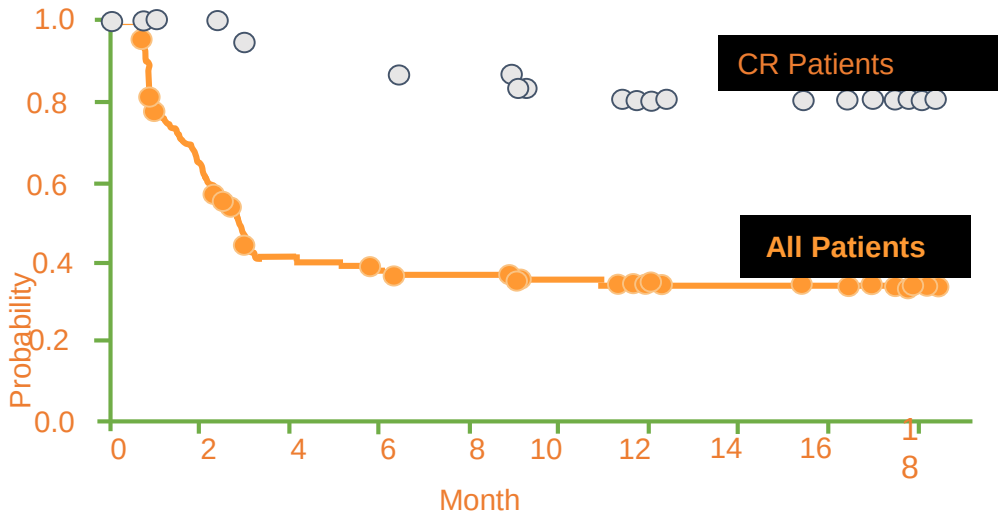


Frey NV, et al. J Clin Oncol. 2019 Dec 9 (epub online)

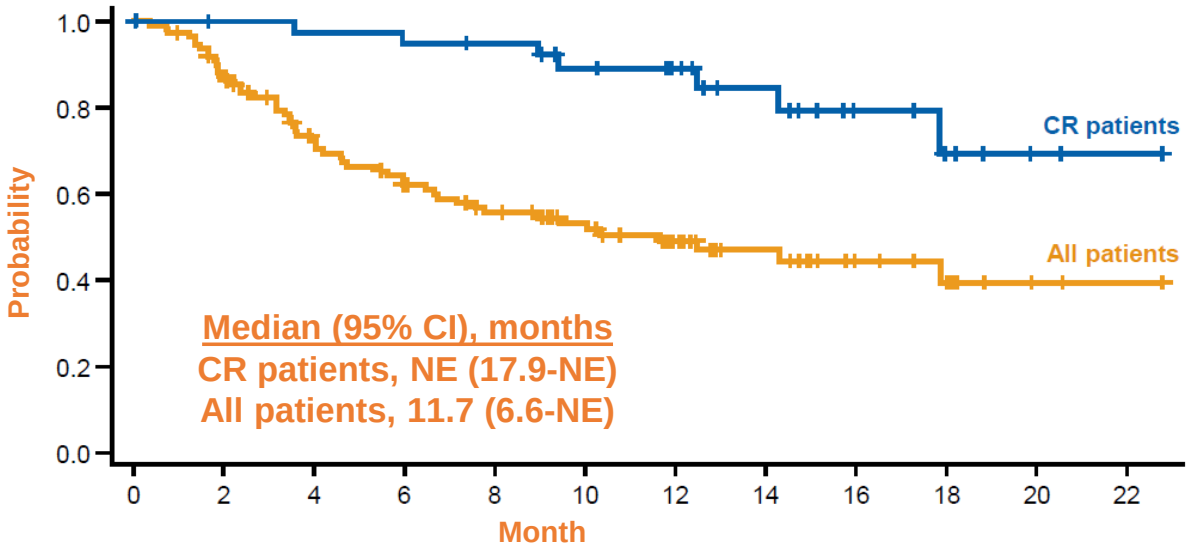
JULIET: Tisagenlecleucel in DLBCL

- Phase II trial of CAR T-cell therapy: **tisagenlecleucel in** 93 adult patients with relapsed or refractory DLBCL

Progression-Free Survival



Overall Survival

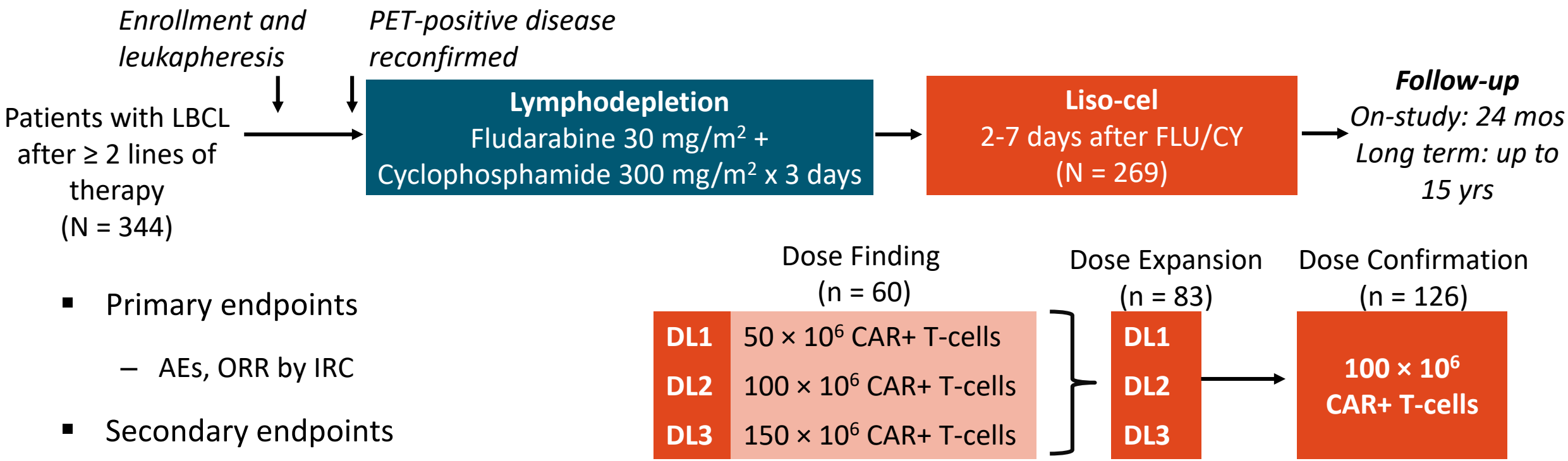


Response Rate (%)	Best Overall (n = 81)	3 Months (n = 81)	6 months (n = 46)
ORR (CR + PR)	52	38	33
CR	40	32	29
PR	12	6	4

Schuster SJ, et al.
N Engl J Med. 2019;380:45-56.

TRANSCEND NHL 001: Study Design

- Pivotal multicenter phase I study



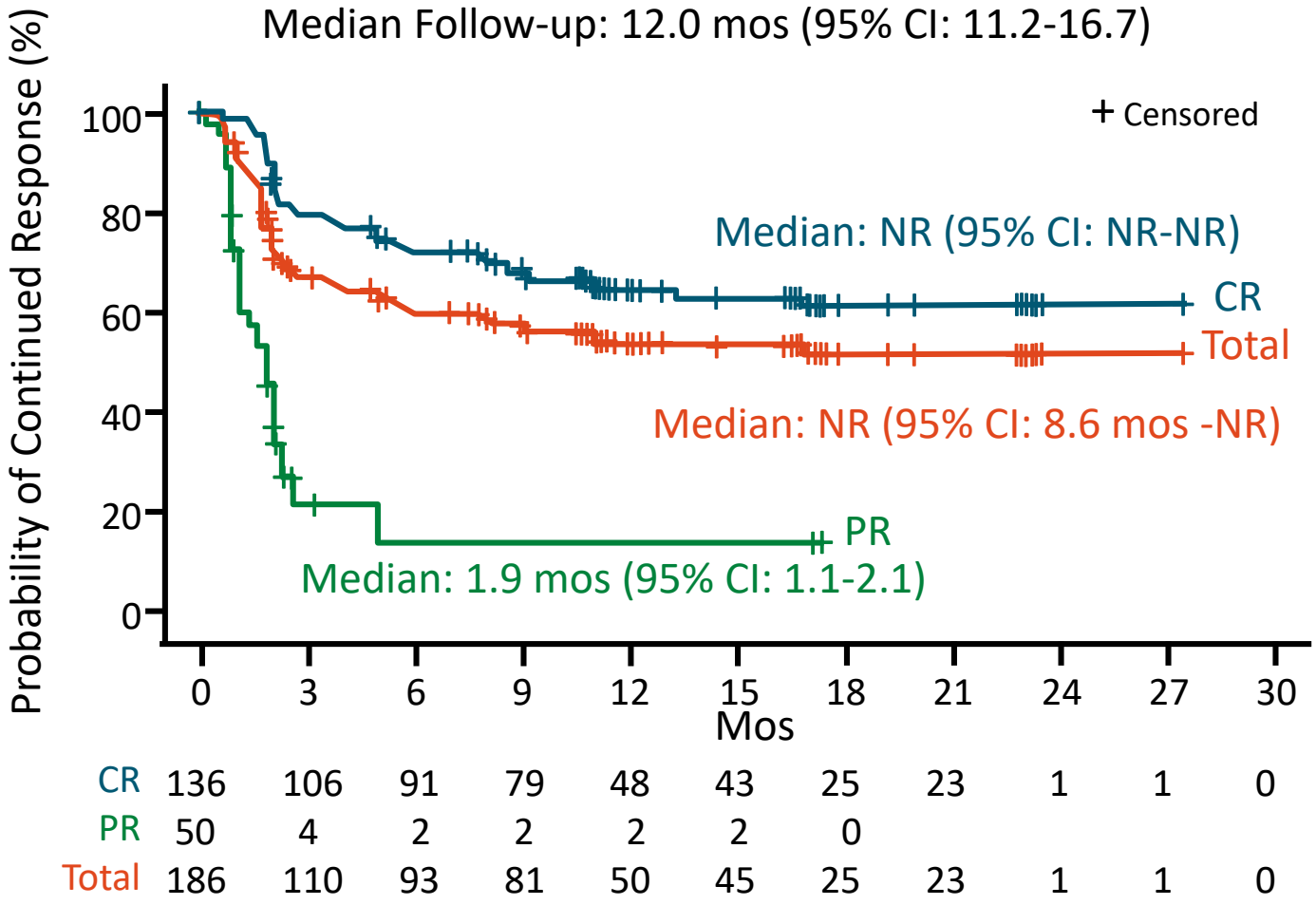
- Primary endpoints
  - AEs, ORR by IRC
- Secondary endpoints
  - CR rate by IRC, DoR, PFS, OS, PK

Abramson. ASH 2019. Abstr 241.

Slide credit: clinicaloptions.com

TRANSCEND NHL 001: Response and Durability by IRC

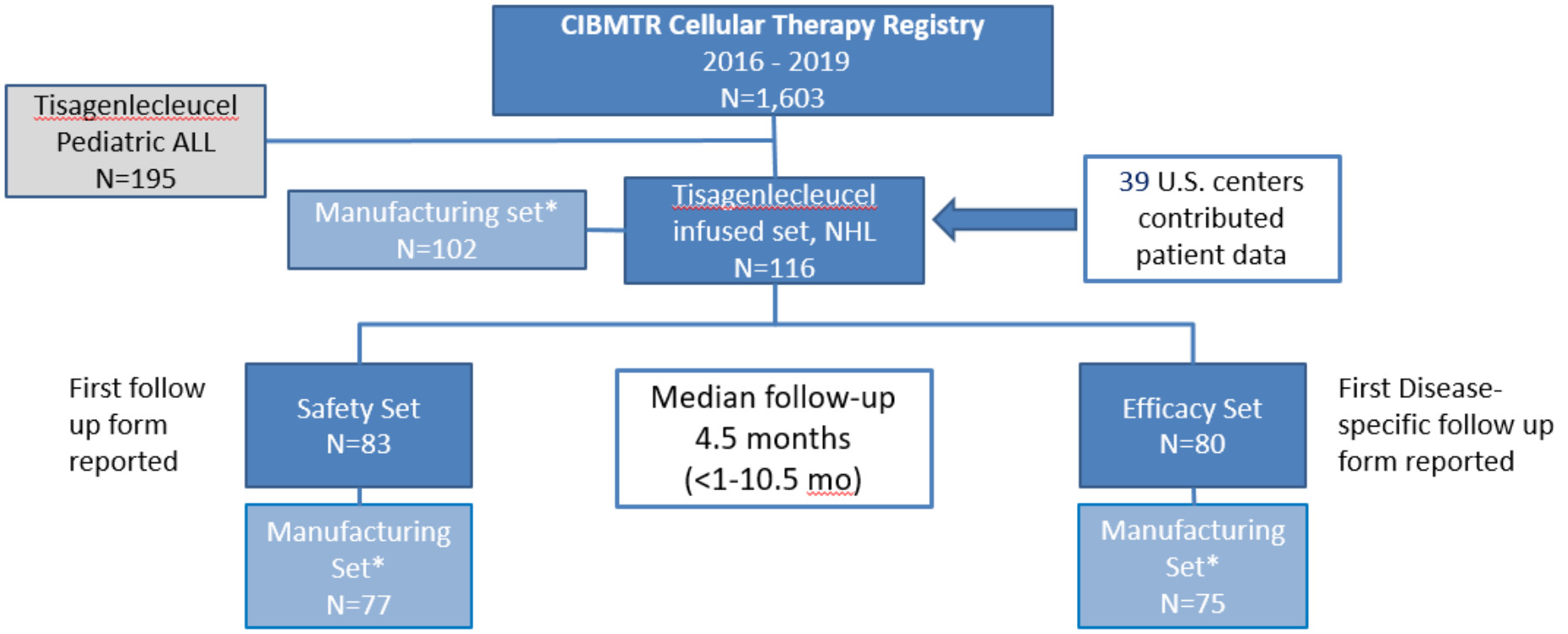
Efficacy-Evaluable Patients (N = 256)	
ORR (95% CI)	73 (67-78)
CR rate (95% CI)	53 (47-59)
Time to first CR or PR, median mos (range)	1.0 (0.7-8.9)
DoR at 6 mos, % (95% CI)	60.4 (52.6-67.3)
DoR at 12 mos, % (95% CI)	54.7 (46.7-62.0)



Abramson. ASH 2019. Abstr 241.

Slide credit: clinicaloptions.com

Overview of Patient Data



Jaglowski, ASH Annual Meeting; 2019; Abstract 766

Baseline Characteristics

Characteristic	NHL (N=116) n (%)
Median Age (range)	65 (15-89)
Male / Female	70 (60) / 46 (40)
Double/triple hit lymphoma	48 (41)
Transformed lymphoma	31 (27)
Disease status prior to tisagenlecleucel	
Refractory/Relapsed	37 (32) / 71 (61)
Prior autologous / allogeneic HCT	28 (24) / 5 (4)
Time from diagnosis to CAR T therapy (median)	15 months
Time from manufacturing start to infusion (median)	32 days

Jaglowski, ASH Annual Meeting; 2019; Abstract 766

Lymphodepleting Therapy

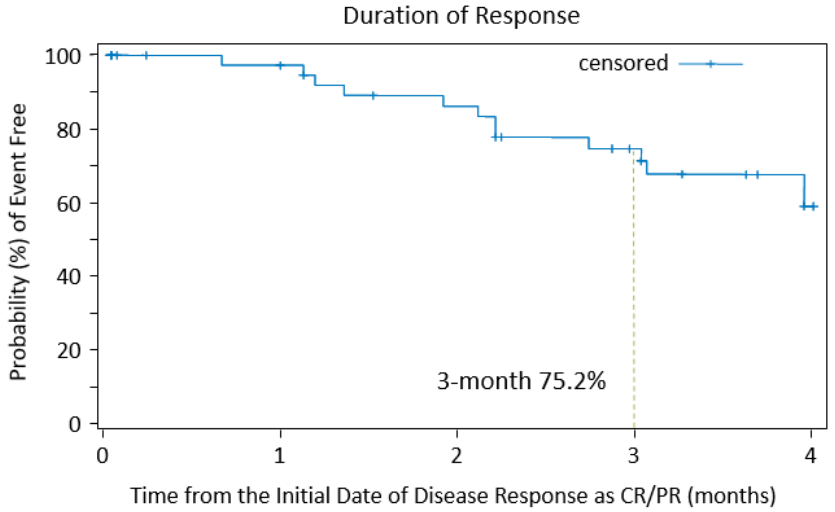
Therapy	NHL (N=116) n (%)
Cyclophosphamide + fludarabine	103 (89)
Bendamustine	6 (5)
Cyclophosphamide + other [a]	4 (4)
Nitrosourea	1 (<1)
Cyclophosphamide	1 (<1)
Not reported	1 (<1)

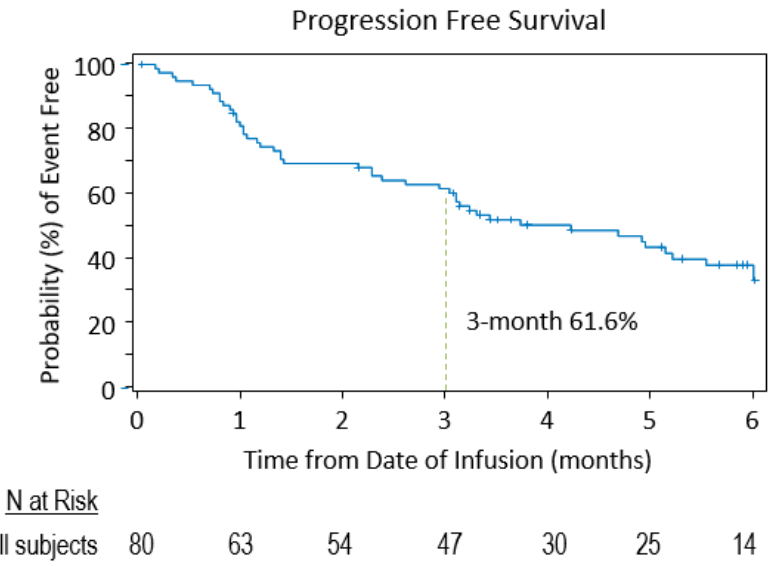
[a]Others: cytarabine, monoclonal antibody

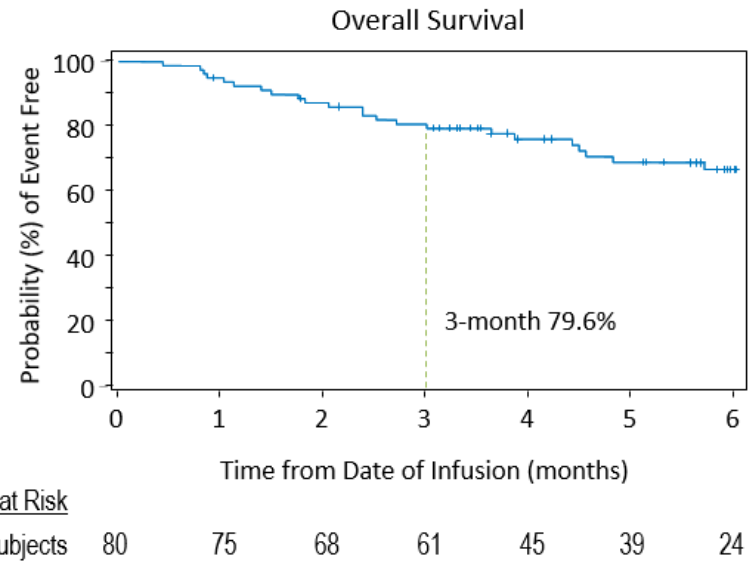
Jaglowski, ASH Annual Meeting; 2019; Abstract 766

Responses and Duration

Response	% (N=80)
Best ORR	58
CR	40
PR	18

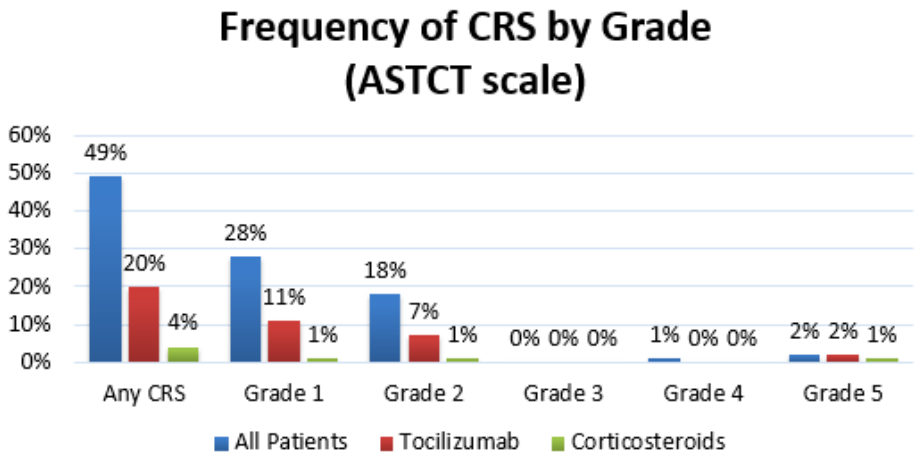


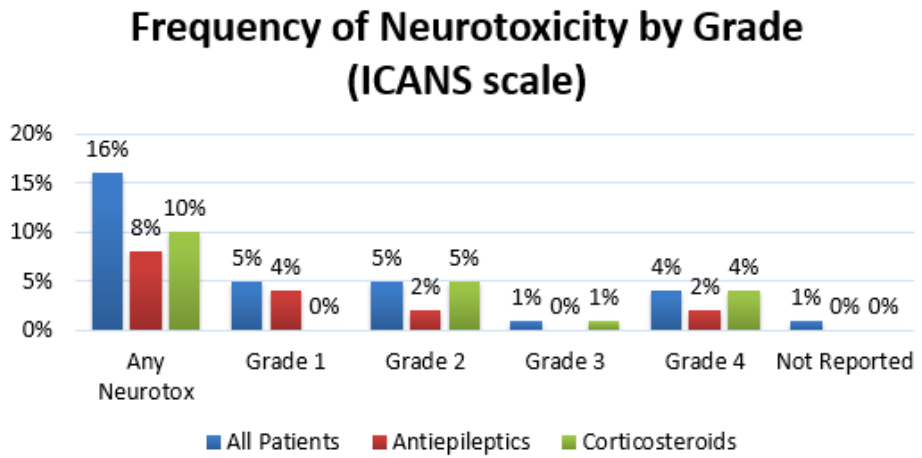




Jaglowski, ASH Annual Meeting; 2019; Abstract 766

Safety





Timing	CRS	Neurotoxicity
Median time to onset in days (range)	4 (2-14)	8 (4-27)
Median duration in days (range)	5 (4-8)	14 (5-25)

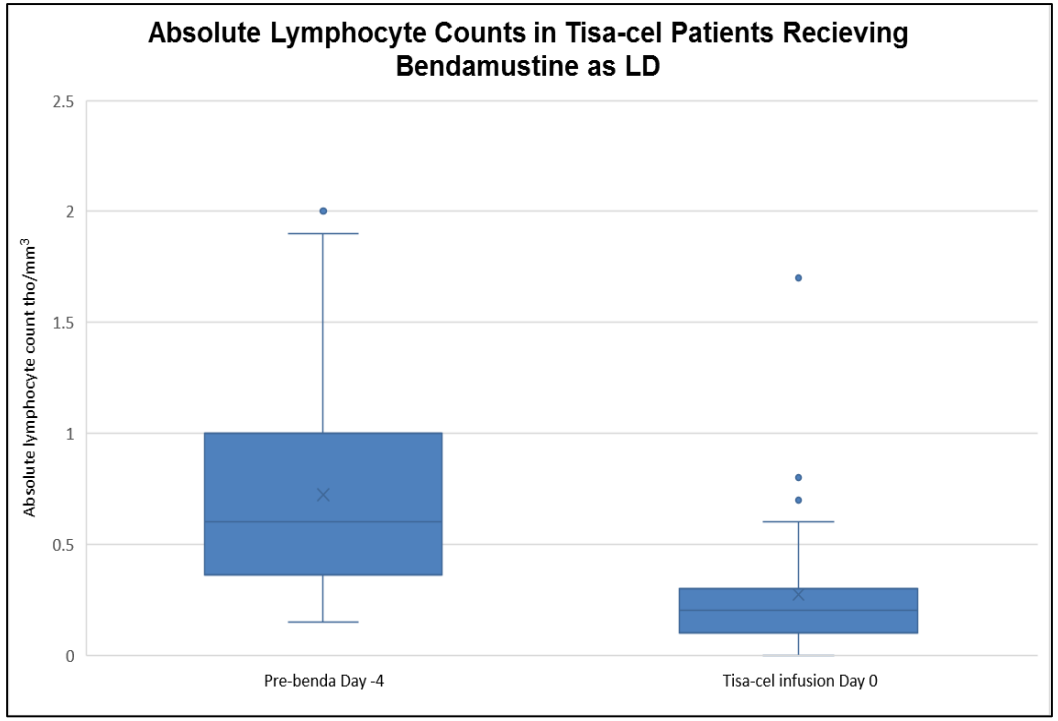
Jaglowski, ASH Annual Meeting; 2019; Abstract 766

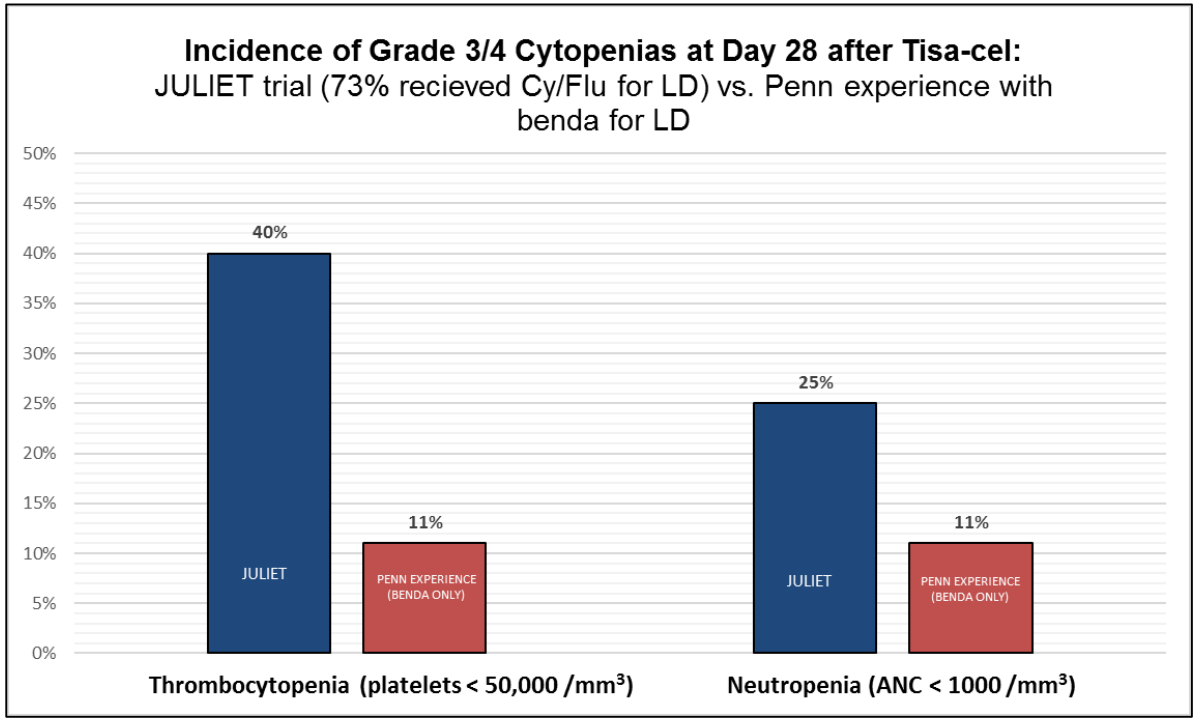
Use of Bendamustine for Lymphodepletion before Tisagenlecleucel (anti-CD19 CAR T cells) for Aggressive B-Cell Lymphomas

- 28 pts received commercially supplied tisa-cel with bendamustine as LD chemotherapy between June 2018 and June 2019
- Bendamustine dose was 90 mg/m2 intravenously daily for 2 days in 23/28 (82%) pts with remaining 5/28 (18%) pts receiving lower doses
- Our institutional experience demonstrates that bendamustine for LD chemotherapy prior tisa-cel:
  - Achieves adequate lymphodepletion
  - Allows outpatient administration
  - Performs well outside of clinical trial settings with comparable response rates to pivotal trials
  - Has a favorable safety profile

Svoboda et al. ASH 2019, Abstract 1606

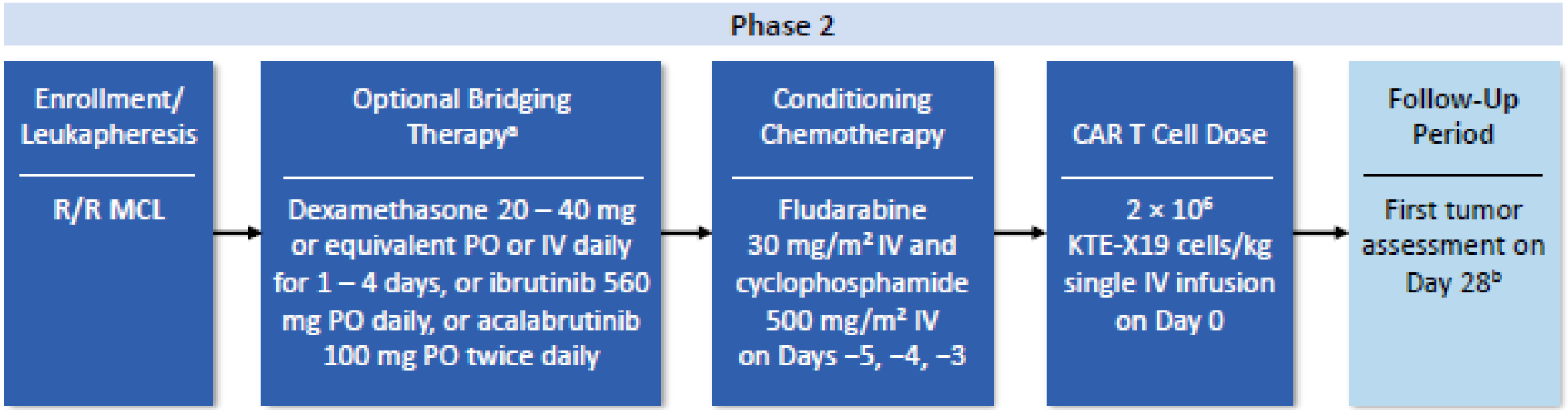
Use of Bendamustine leads to less grade 3/4 cytopenias and more lymphodepletion





Svoboda et al. ASH 2019, Abstract 1606

ZUMA-2 Study Design



Primary Endpoint

- ORR (IRRC-assessed per the Lugano classification[1])

Key Secondary Endpoints

- DOR
- PFS
- OS
- AEs
- ORR (Investigator-assessed per revised IWG criteria[2])
- EQ-5D
- Levels of CAR T cells in blood and cytokines in serum

[a] Administered after leukapheresis and completed ≤ 5 days before initiating conditioning chemotherapy; PET-CT was required post-bridging.
[b] Bone marrow biopsy was done at screening and if positive, not done, or indeterminate, a biopsy was needed to confirm CR.
AE, adverse event; CAR, chimeric antigen receptor; DOR, duration of response; EQ-5D, European Quality of Life-5 Dimensions; IRRC, Independent Radiology Review Committee; IWG, International Working Group; MCL, mantle cell lymphoma; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PO, oral; R/R, relapsed/refractory.
1. Cheson BD, et al. *J Clin Oncol*. 2014;32:3059-3068. 2. Cheson BD, et al. *J Clin Oncol*. 2007;25:579-586.

ZUMA-2 Patient Eligibility

Key Inclusion Criteria

- R/R MCL defined as
  - Disease progression after last regimen or
  - Failure to exhibit a CR or PR to the last regimen
- 1 – 5 Prior therapies that must have included
  - An anthracycline- or bendamustine-containing chemotherapy and
  - Anti-CD20 monoclonal antibody therapy and
  - Ibrutinib or acalabrutinib
- ≥ 1 Measurable lesion
- Age ≥ 18 years
- ECOG of 0 or 1
- Adequate bone marrow, renal, hepatic, pulmonary, and cardiac function
- ALC ≥ 100/µL

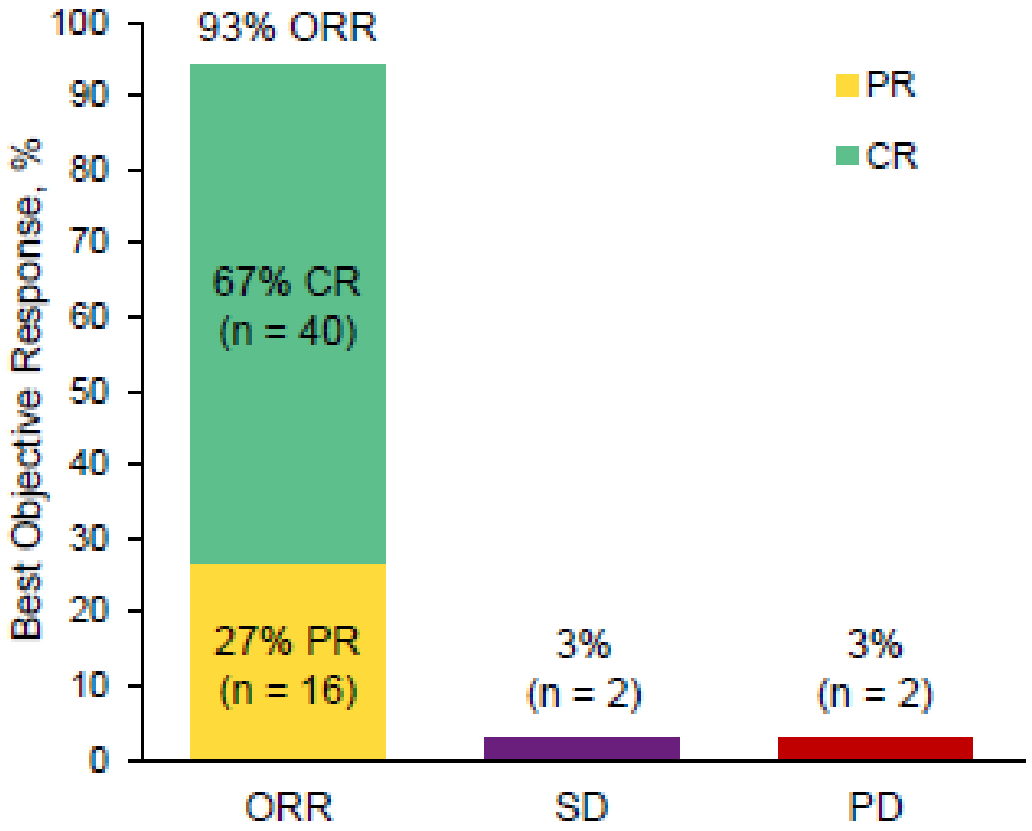
Key Exclusion Criteria

- Prior allogeneic SCT
- Prior CD19-targeted therapy
- Prior CAR T cell therapy
- Clinically significant infection
- History of or current CNS involvement by MCL or other CNS disorders

Characteristic	N = 68
Median no. of prior therapies (range)[a]	3 (1 – 5)
≥ 3, n (%)	55 (81)
Anthracycline or bendamustine, n (%)	67 (99)
Anti-CD20 mAb, n (%)	68 (100)
Relapsed after autologous SCT	29 (43)
BTKi, n (%)	68 (100)
Ibrutinib[b]	58 (85)
Acalabrutinib	16 (24)
Both	6 (9)
BTKi refractory, n (%)	46 (68)
Refractory to ibrutinib	38 (56)
Refractory to acalabrutinib	8 (12)
BTKi relapsed, n (%)	22 (32)
Relapse while on drug	14 (21)
Relapse > 30 days after discontinuing drug	5 (7)
Intolerant but with evidence of PD	3 (4)

CAR, chimeric antigen receptor; CNS, central nervous system; CR, complete response; ECOG, Eastern Cooperative Oncology Group performance status; MCL, mantle cell lymphoma; PR, partial response; R/R, relapsed/refractory; SCT, stem-cell transplant.

ORR by IRRC Assessment Was 93% (95% CI, 84 – 98) and CR Rate Was 67% (95% CI, 53 – 78)



	Efficacy-Evaluable N = 60
Median follow-up (range), mo	12.3 (7.0 – 32.3)
Patients with ≥ 24 mo follow-up, n (%)	28 (47)
Median time to response (range), mo	
Initial response	1.0 (0.8 – 3.1)
CR	3.0 (0.9 – 9.3)
Patients converted from PR/SD to CR, n (%)	24 (40)
PR to CR	21 (35)
SD to CR	3 (5)

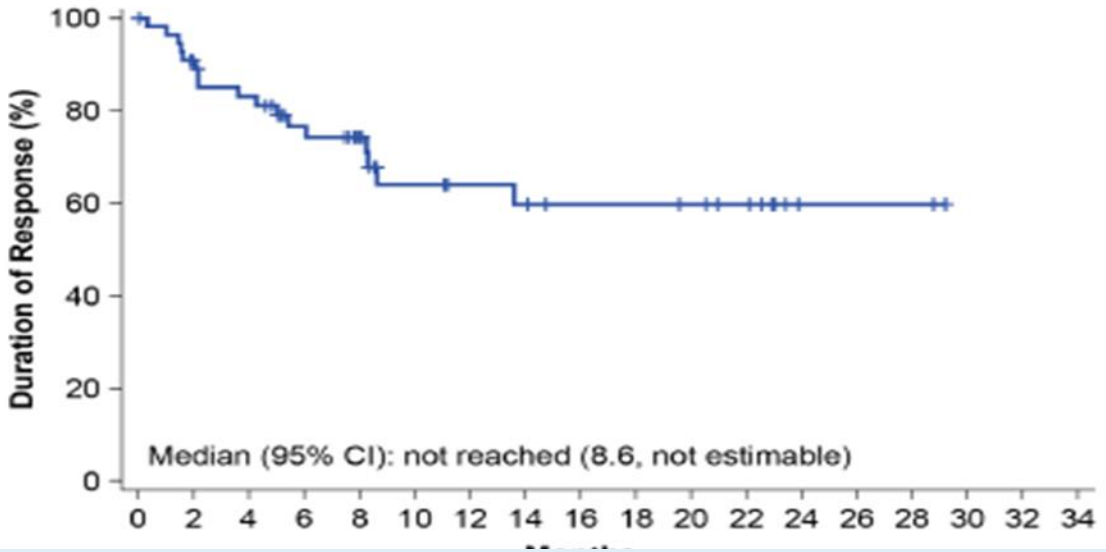
Investigator-assessed ORR in N = 60 was 88% (CR rate 70%), with 95% and 90% concordance between IRRC- and investigator-assessed ORR and CR rate, respectively. IRRC-assessed ORR in ITT (N = 74) was 85% (CR Rate 59%).
CR, complete response; IRRC, Independent Radiology Review Committee; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

ASH 2019 Abstract 754, Courtesy of Michael Wang

Duration of Response

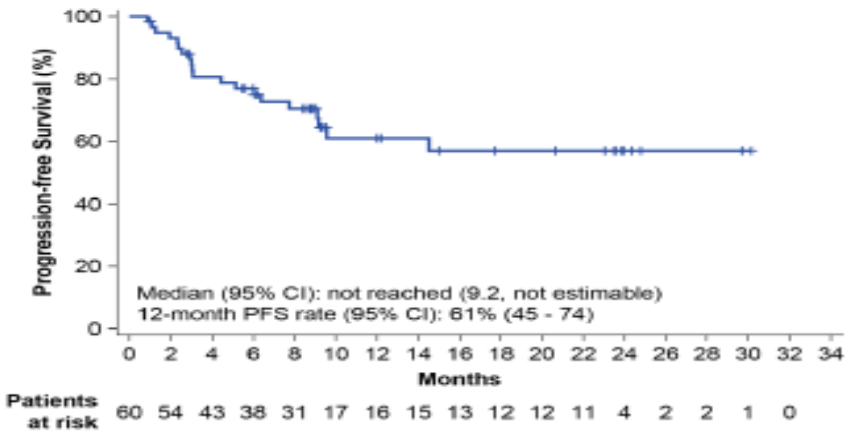
- The median DOR has not been reached after a median follow-up of 12.3 months
  - 57% of all patients and 78% of patients with a CR remain in remission
- The first 28 patients treated had a median follow-up of 27.0 months (range, 25.3 – 32.3)
  - 43% remain in continued remission without additional therapy

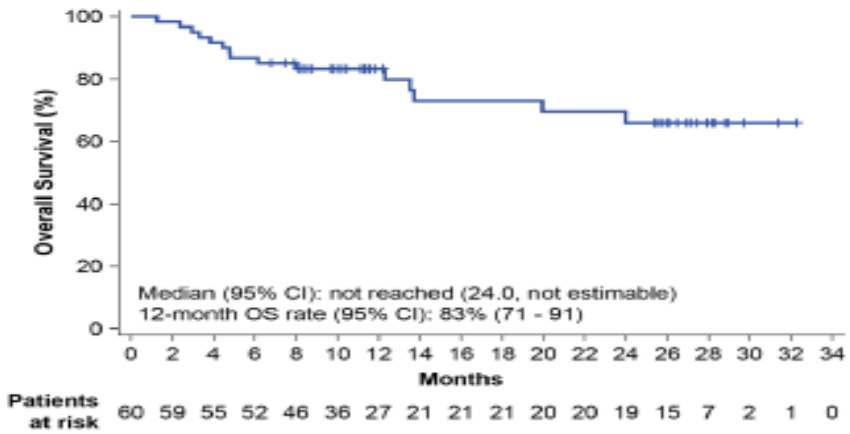
CR, complete response; DOR, duration of response.



Progression-Free Survival and Overall Survival

- Median PFS and median OS were not reached after a median follow-up of 12.3 months





OS, overall survival; PFS, progression-free survival.

BCMA CAR T cells – initial studies, refractory pts

Trial	n	CAR	Condi-tioning	# lines	% hi risk†	Dosing	ORR	ORR (optimal doses)	VGPR/CR (optimal doses)
NCI[1]	26*	Murine, CD3/CD28	Cy/Flu	7.5	42%	0.3 – 9 x 10^6/kg	58%	81% (13/16)	63% (10/16)
Penn[2]	25	Human, CD3/41BB	None or Cy	7	76%	0.5 – 5 x 10^8	48%	64% (7/11)	36% (4/11)
Bluebird[3]	43	Human, CD3/41BB	Cy/Flu	7.5	40%	0.5 – 8 x 10^8	77% (30/39)	96% (21/22)	86% (19/22)

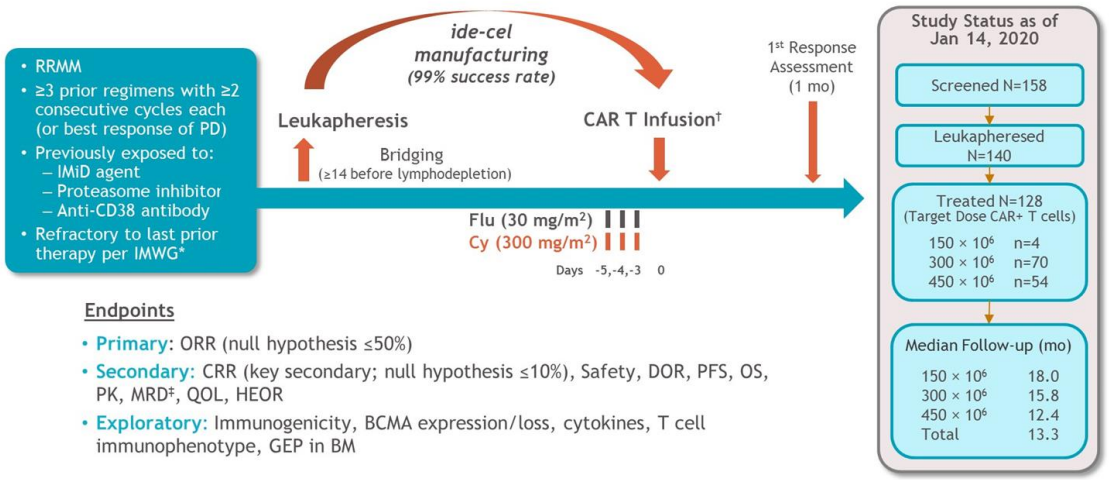
***2 treated twice; counted separately for response. † FISH +t(4;14), t(14;16), del 17p**

Trial	n	CRS %	CRS G3-4 %	Neuro tox %	Neuro tox G3-4 %	Tocilizumab
NCI[1]	26*	73%	23%	NR	12%	19%
Penn[2]	25	88%	32%	32%	12%	28%
Bluebird[3]	43	63%	5%	33%	2%	21%

***excluded high tumor burden in last 14 pts. NR = not reported**

BCMA Directed CAR T Studies: ASH 2019, ASCO 2020

Phase II Pivotal KarMMa Study



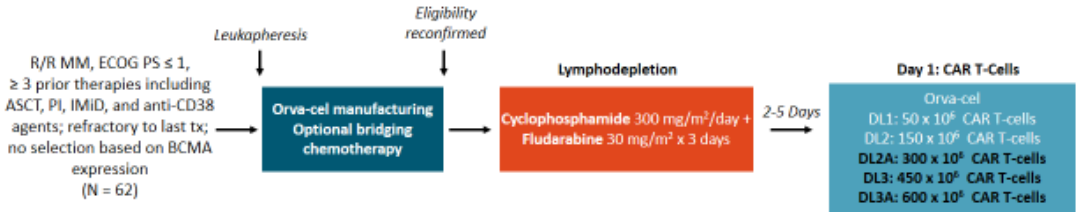
Endpoints

- **Primary:** ORR (null hypothesis ≤50%)
- **Secondary:** CRR (key secondary; null hypothesis ≤10%), Safety, DOR, PFS, OS, PK, MRD‡, QOL, HEOR
- **Exploratory:** Immunogenicity, BCMA expression/loss, cytokines, T cell immunophenotype, GEP in BM

CRR, complete response rate; Cy, cyclophosphamide; DOR, duration of response; Flu, fludarabine; GEP in BM, gene expression profile in bone marrow; HEOR, health economics and outcomes research; IMiD, immunomodulatory drug; IMWG, International Myeloma Working Group; MRD, minimal residual disease; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PK, pharmacokinetics; QOL, quality of life.
*Defined as documented disease progression during or within 60 d from last dose of prior antimyeloma regimen. †Patients were required to be hospitalized for 14 d post-infusion. Ide-cel retreatment was allowed at disease progression for best response of at least stable disease. ‡By next-generation sequencing.

EudraCT: 2017-002245-29
ClinicalTrials.gov: NCT03361748

EVOLVE: Study Design



- Primary objectives (phase I): safety and tolerability (DLTs, AEs) and RP2D
- Secondary objectives (phase I): orva-cel pharmacokinetics, preliminary antitumor activity

Mailankody. ASCO 2020. Abstr 8504.

CARTITUDE-1: Phase 1b/2 Study Design

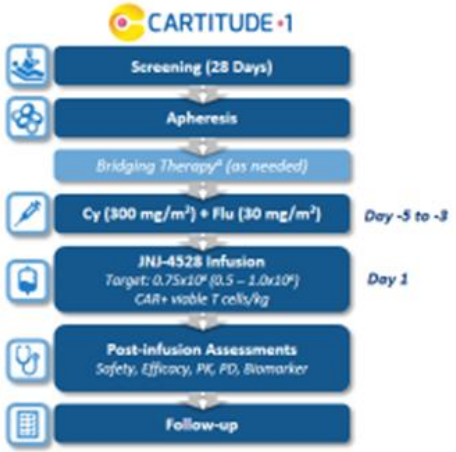
Primary Objectives

- **Phase 1b:** Characterize safety and confirm phase 2 dose as informed by the LEGEND-2 study
- **Phase 2:** Evaluate efficacy of JNJ-4528

Key Eligibility Criteria

- Progressive MM per IMWG criteria
- ECOG PS ≤1
- Measurable disease
- Received ≥3 prior therapies or double refractory
- Prior PI, IMiD, anti-CD38 therapy

- Median administered dose = 0.73x10⁶ (0.52 – 0.89x10⁶) CAR+ viable T cells/kg
- Median follow-up at data cut-off = 6 mo (3 – 14)



Similar approach in 3 studies:

R/R MM
Steady state T cell collection
CY/FLU lymphodepletion
Single infusion

BCMA Directed CAR T Studies: ASH 2019, ASCO 2020

Patient Characteristics

	KarMMa: idecabtagene vicleucel (n=128)	EVOLVE: orvacabtagene autoleucel (n=62)	CARTITUDE-1: JNJ-4528 (n = 29)
Age	61 (33-78)	61 (33-77)	60 (50-75)
High Risk Cytogenetics, %	35	41*	27
Tumor Burden in BM, %	>50% PC = 51	—	≥60% PC = 24
Extramedullary PCs, %	39	23	10
Median prior lines of therapy	6 (3-16)	6 (3-18)	5 (3-18)
Triple refractory, %	84	94	86
Bridging therapy, %	88	63	79
Unique properties	Human BCMA, 4-1BB, CD3z	Modified spacer, CD4:CD8 enriched for CM	Median cell dose **0.72x10^6** cells/kg 2 BCMA single chain antibodies

BCMA Directed CAR T Studies: ASH 2019, ASCO 2020

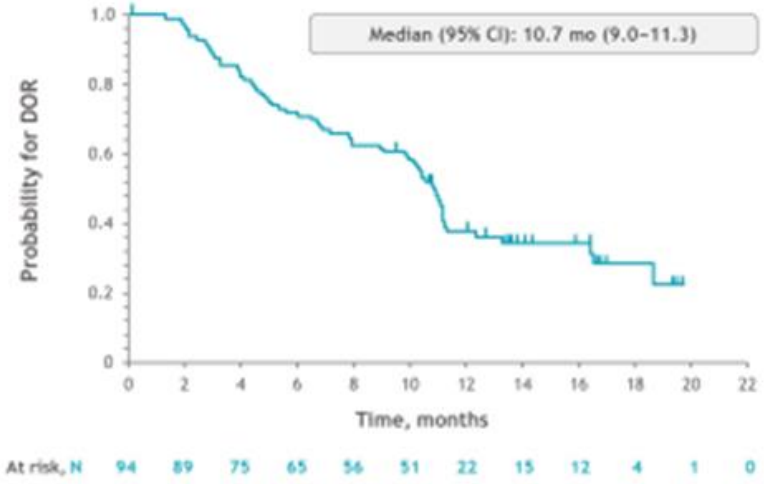
Response Rates

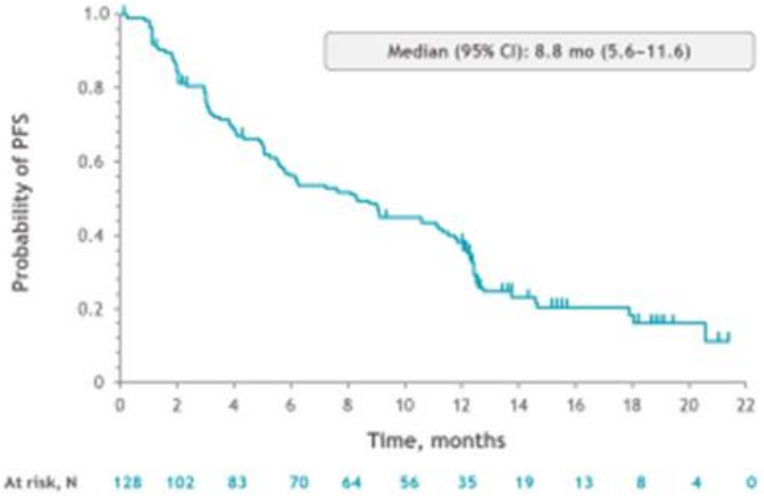
	KarMMa	EVOLVE	CARTITUDE-1
↓ANC ≥G3, %	89	90	100
↓plts ≥G3, %	52	47	69
CRS: all, ≥G3,%	84, 6	89, 3	93, 7
Med. time to CRS, duration, days	1 (1-12) 5 (1-63)	2 (1-4) 4 (1-10)	7 (2-12) 4 (2-64)
ICANS: all, ≥G3,%	17, 3	13, 3	10, 3
HLH/MAS, %	--	5	? 7 (lfts)
Infections: all, ≥G3 %	69, --	40, 13	--, 19
Toci/steroid/ anakinra use, %	52/15/0	76/52/23	79/21/21

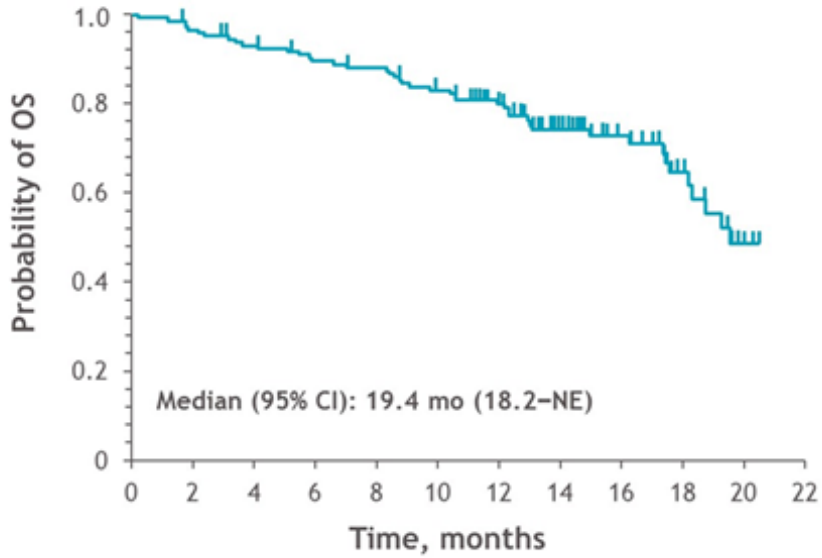
	KarMMa (n = 128)	EVOLVE (n = 62)	CARTITUDE-1 (n = 29)
ORR, %	73 (66-81)	92	100
sCR/CR, %	33	36	86
MRD neg ≥10^{-5}, % (of evaluable)	94	84	81
PFS/DoR, months	8.8/10.7	NR*	NR**
Screened Apheresed Treated	150 140 128	--	35 35 29

BCMA Directed CAR T Studies: KarMMa

Duration of Response, Progression Free and Overall Survival

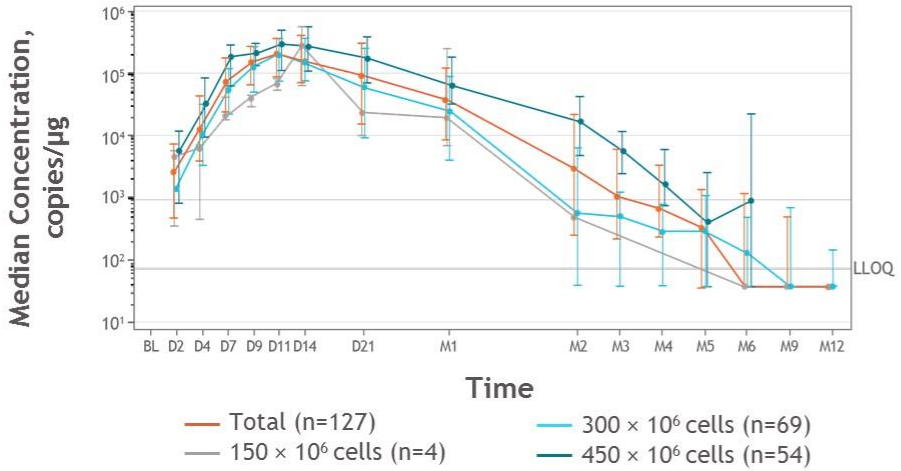






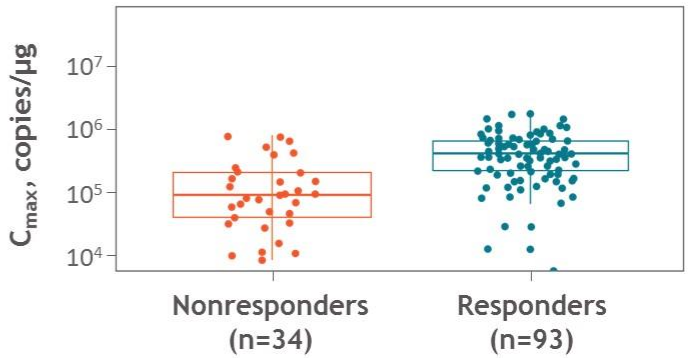
CAR+ T Cell Expansion, Persistence, and Peak Exposure

CAR+ T Cell Expansion and Persistence



	Mo 1	Mo 3	Mo 6	Mo 9	Mo 12
Evaluable patients, n	118	100	49	27	11
Patients with detectable vector, n (%)	117 (99)	75 (75)	29 (59)	10 (37)	4 (36)

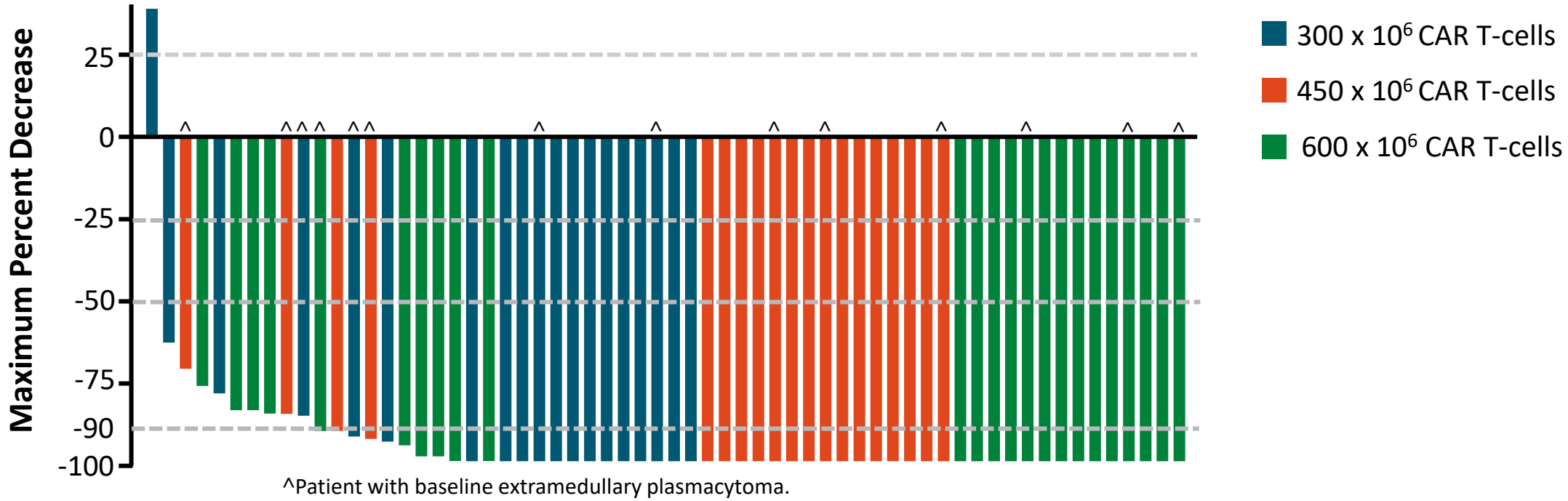
Peak Vector Copies in Responders (≥PR) vs Nonresponders (<PR)



- Median peak CAR+ T cell expansion was at 11 d
- Median expansion increased at higher target doses with overlapping profiles
- Peak exposure higher in responders than nonresponders
- Durable persistence was observed up to 1 y

Data cutoff: 19 April 2019. Pharmacokinetic (PK) analysis population (N=127). One patient died on day 4 and had no evaluable PK samples and was therefore excluded. Error bars represent interquartile range. BL, baseline; C_{max}, maximum concentration; LLOQ, lower limit of quantitation; M, month.

EVOLVE: Tumor Burden Reduction According to Dose

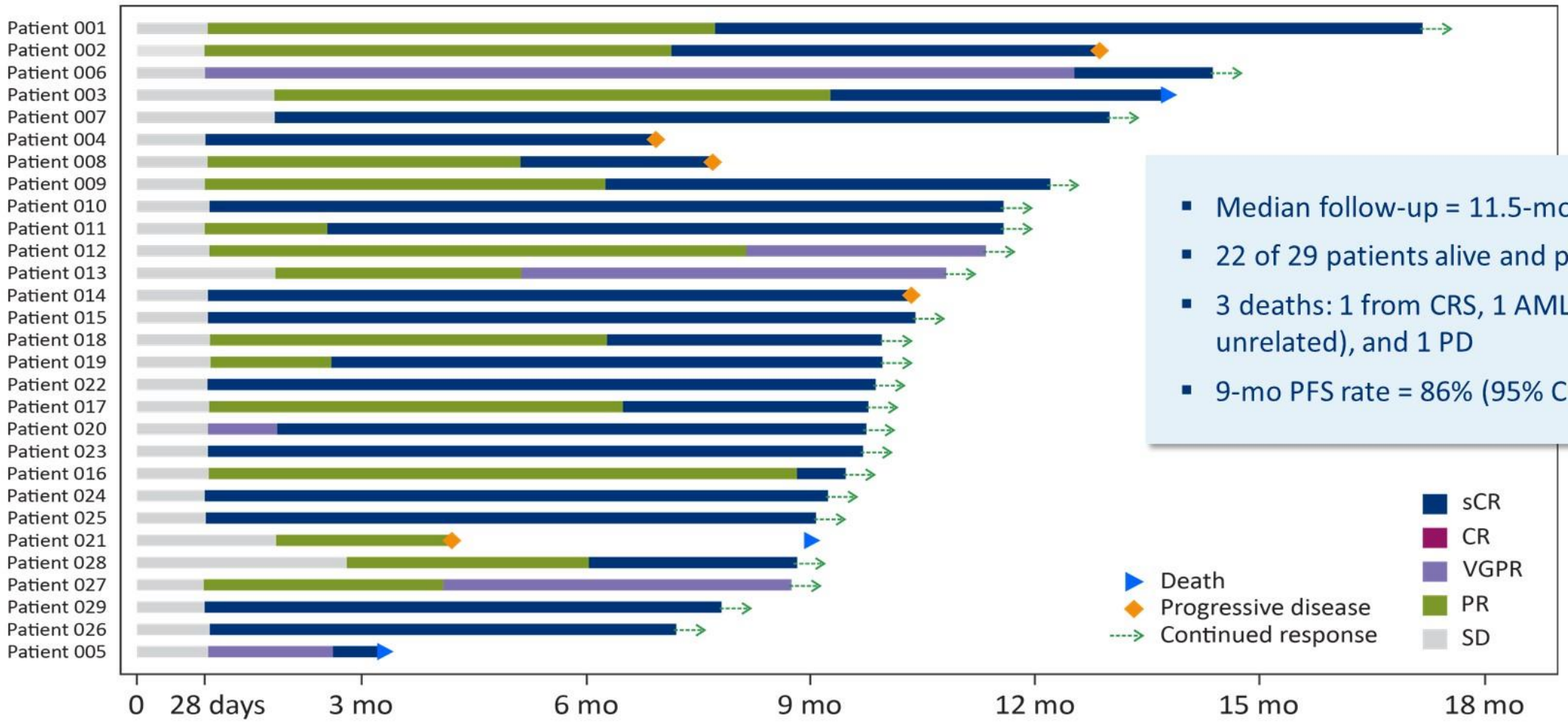


^Patient with baseline extramedullary plasmacytoma.

- Serologic responses (serum or urine paraprotein, free light chains) were observed in all patients treated at 450 x 10^6 and 600 x 10^6 dose levels
- Orva-cel activity not impacted by high baseline sBCMA
  - 12/12 patients achieved ≥ PR; 8/12 ≥ VGPR

Mailankody. ASCO 2020. Abstr 8504..

CARTITUDE-1: Duration of Response



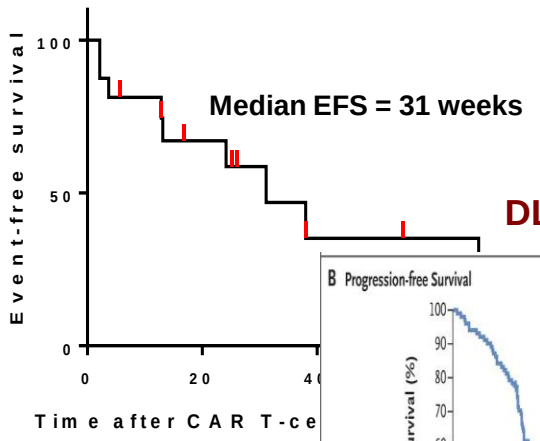
- Median follow-up = 11.5-mo (3 – 17)
- 22 of 29 patients alive and progression-free
- 3 deaths: 1 from CRS, 1 AML (treatment unrelated), and 1 PD
- 9-mo PFS rate = 86% (95% CI, 67 – 95)

AML=acute myeloid leukemia (biphenotypic); PD=progressive disease; PFS=progression-free survival

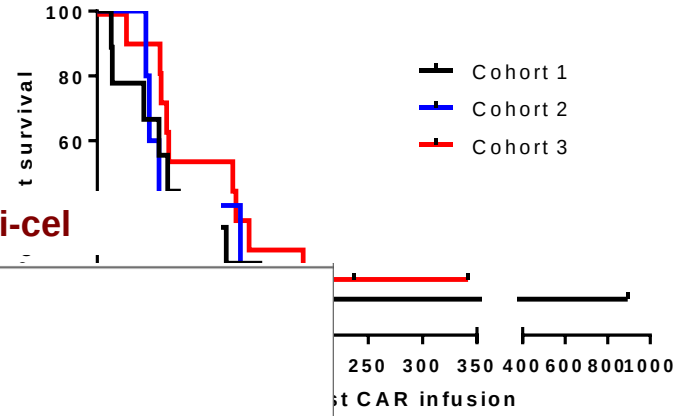
BCMA CAR T cells – lessons from initial studies

- **Probably not curative in refractory patients**

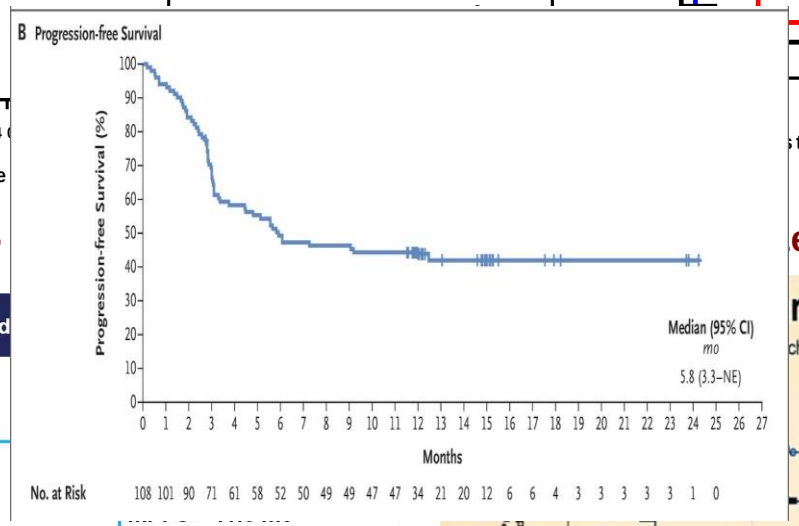
NCI – 9x10e6/kg



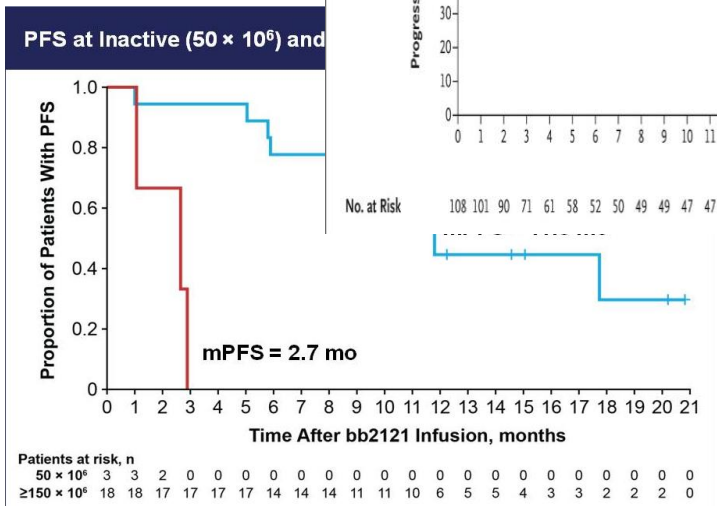
Penn



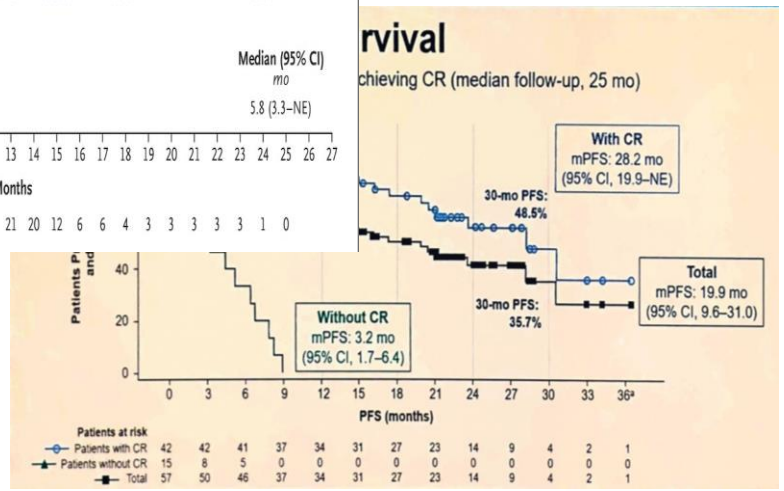
DLBCL ph2 Axi-cel



Bluebird –



egend



Why not more durable responses?

- **CAR-intrinsic factors**
  - Binding affinity, epitopes
  - Tonic signaling
  - Co-stimulation
- **T-cell intrinsic factors**
  - Pre-manufacturing
  - Post-manufacturing
  - Post-infusion
- **Tumor-intrinsic factors**
  - Myeloma cell
  - Microenvironment
- **Other**
  - Lymphodepletion regimen?

Cohen et al, J Clin Invest 2019; Brudno et al, J Clin Oncol 2018; Wang et al, ASH 2019, #1909

Background

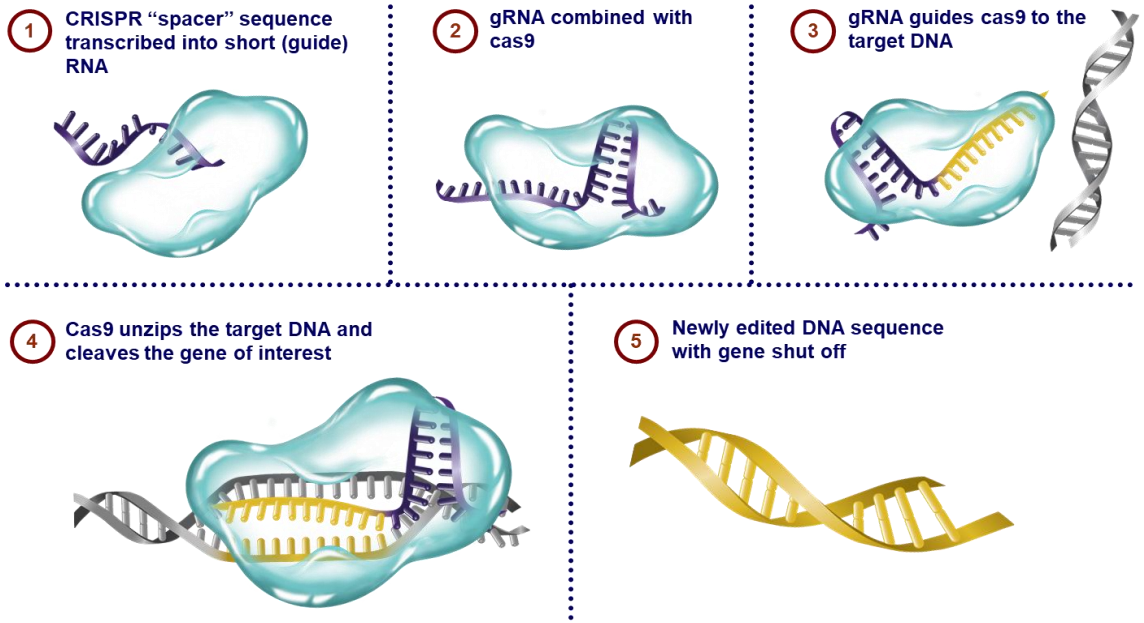
- **Autologous T cells genetically modified with a lentiviral vector to express affinity-enhanced T cell receptors (TCRs) or chimeric antigen receptors (CAR T cells) have shown great promise for the treatment of cancer**

- **Unfortunately, CAR T is not always successful. Lack of response to adoptive T cell therapy is due in some cases to intrinsic autologous T cell defects, poor expansion and persistence or the inability of these cells to function optimally in a strongly immunosuppressive tumor microenvironment**

- **By combining the tools of synthetic biology such as TCRs and CRISPR/Cas9, we have an unprecedented opportunity to optimally program T cells and improve adoptive immunotherapy**

Big Questions in the Field

- **Is it <u>feasible</u> to generate multiplexed CRISPR-edited T cells at the scale needed for a clinical infusion product?**
- **Can patients receive such a T cell product <u>safely</u> following lymphodepletion?**
- **Will these cells <u>expand, persist and elicit anti-tumor activity</u> in patients?**



American Society of Hematology
61st Annual Meeting & Exposition

First-in-Human Assessment of Feasibility and Safety of Multiplexed Genetic Engineering of Autologous T Cells Expressing NY-ESO -1 TCR and CRISPR/Cas9 Gene Edited to Eliminate Endogenous TCR and PD-1 (NYCE T cells) in Advanced Multiple Myeloma (MM) and Sarcoma

Edward Stadtmauer, MD, Adam Cohen, MD, Kristy Weber, MD, Simon Lacey, PhD, Vanessa Gonzalez, J. Joseph Melenhorst, PhD, Joseph Fraietta, PhD, Gabriela Plesa, MD, PhD, Joanne Shea, Tina Matlawski, RN, Amanda Cervini, RN, Patricia Mangan, CRNP, Avery Gaymon, Stephanie Desjardins, Eric Lancaster, MD, January Salas-Mckee, Jun Xu PhD, Megan Suhoski, PhD, Andrew Fesnak, MD, Matthew O'Rourke, Anne Lamontagne, Don Siegel, MD, PhD, Regina Young, PhD, Anne Chew, PhD, Christopher Nobles, PhD, Frederic Bushman, PhD, Howard Chang, MD, PhD, Ansuman Satpathy, MD, PhD, Yangbing Zhao, PhD, MD, Bruce Levine, PhD, Wei-Ting Hwang, PhD, Elizabeth Hexner, MD, MS and Carl June, MD

Division of Hematology-Oncology, Department of Medicine
Departments of Orthopedic Surgery, Microbiology, Neurology and Pathology
Center for Cellular Immunotherapies
Abramson Cancer Center, University of Pennsylvania
Philadelphia, PA, USA

ASH December 7, 2019 and *Science* February 28, 2020

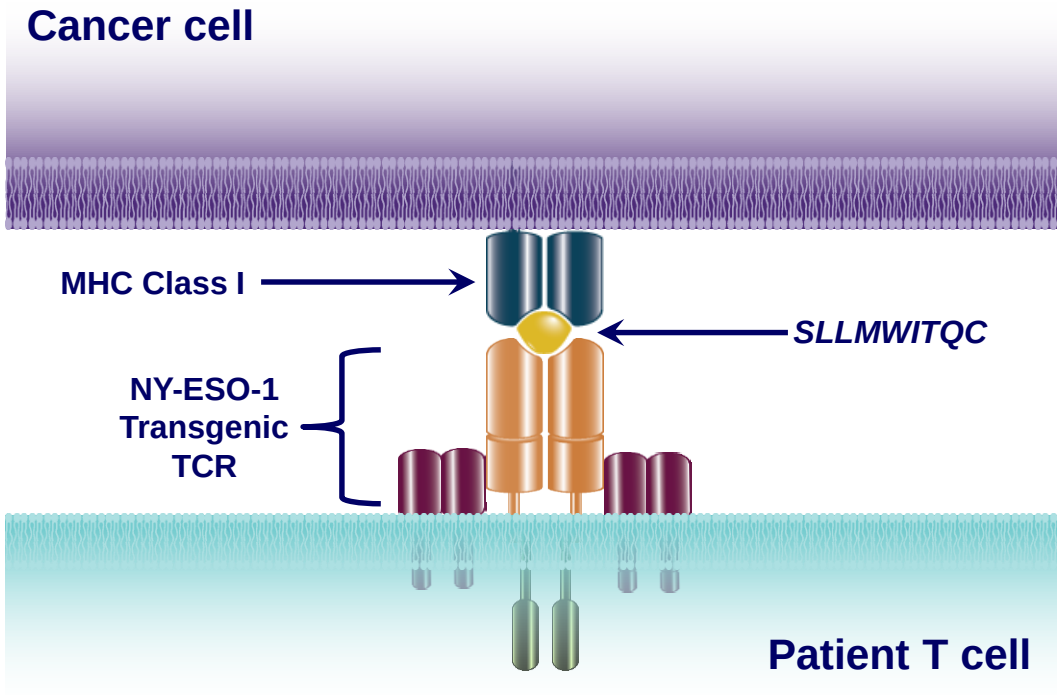
Penn Medicine
Center for Cellular Immunotherapies

Penn Medicine
Abramson Cancer Center

Generation of a Novel NY-ESO-1 Transgenic T cell Receptor (TCR)

- Expression of NY-ESO-1 and LAGE-1, cancer testis antigens, is limited to a variety of cancers and germ cells of the testis
- NY-ESO-1 and LAGE-1 genes encode very homologous proteins (common SLLMWTTQC epitope)
- Studies of NY-ESO-1 TCR-expressing T cells show safety and evidence of anti-tumor activity in melanoma, sarcoma and myeloma
- Lack of long-term durable responses underscores the need to improve the clinical efficacy of this approach

- We generated a novel transgenic TCR
  - Targets NY-ESO-$1_{157\text{-}165}$
  - Derived from a cytotoxic T lymphocyte clone (TE8-1-8F)
  - Constructed using overlapping PCR
  - Requires HLA-A*0201 restriction

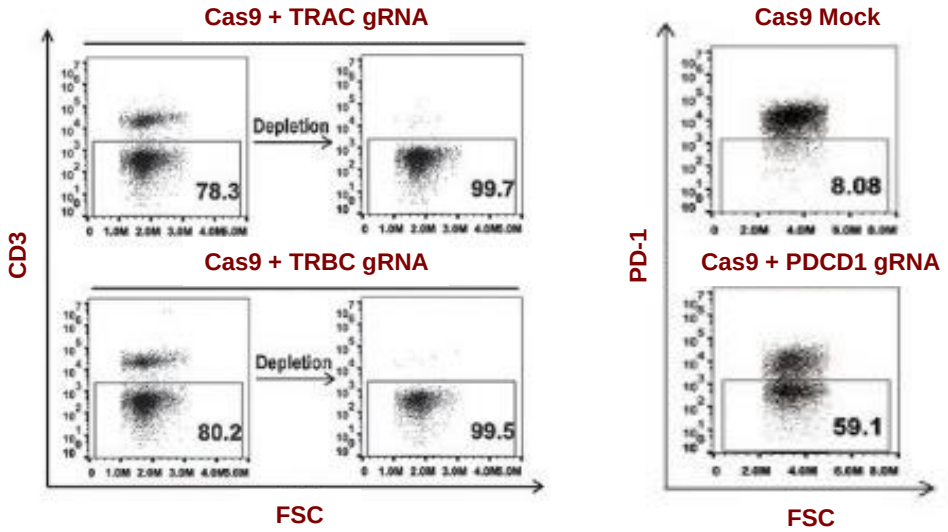


Rapoport et al, *Nat. Med.* 2015; Stadtmauer et al, *Blood Adv.* 2019;
Robbins et al, *Clin. Cancer Res.* 2015; D'Angelo, et al, *Can. Discov.* 2018

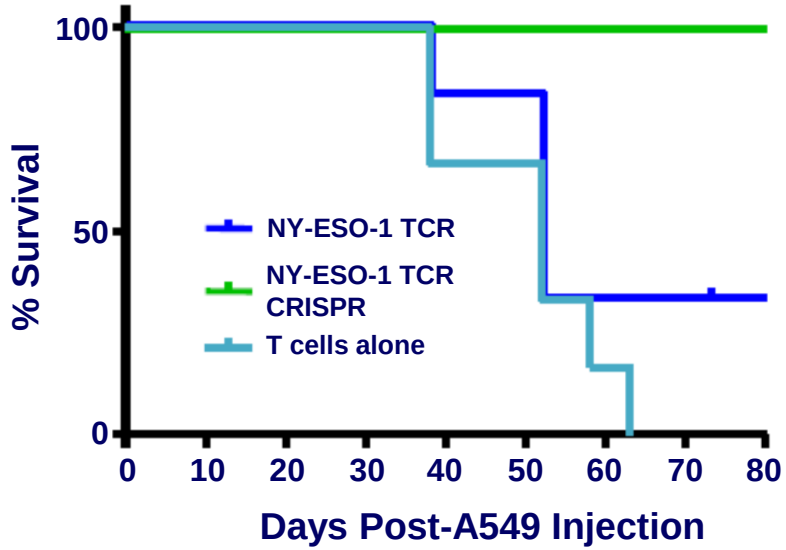
Hypothesis and Pre-clinical Work

- We hypothesized disease progression despite T cell therapy was associated with T cell exhaustion and lack of T cell persistence, which may be mediated by PD-1
- Therefore, knock-out of 3 genes would enhance the efficacy and persistence of the NY-ESO-1 directed T cells
  - Removal of genes encoding the endogenous TCR, TCRα (*TRAC*) and TCRβ (*TRBC*), would reduce TCR mispairing, thereby enhancing NY-ESO-1 TCR activity and preventing autoimmunity
  - Removal of PD-1 (*PDCD1*) would increase expansion and anti-tumor potency, while reducing T cell hypofunction
- We previously demonstrated CRISPR/Cas9 and *TRAC*, *TRBC* and *PDCD1* targeting gRNAs could be successfully introduced via electroporation in preclinical models to disrupt gene expression

CRISPR/Cas9 Editing of T cells



Overall Survival



Ren et al, *Clin. Cancer Res.* 2016

Clinical Study Objectives

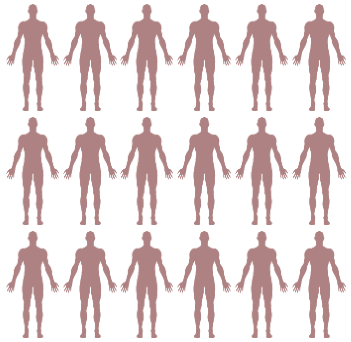
- **Primary:**
  - **Determine safety profile of a single infusion of NYCE T cells**
  - **Evaluate manufacturing feasibility of NYCE T cells**

- **Secondary Clinical:**
  - **Describe anti-tumor responses and survival after infusion in advanced cancer**

- **Secondary Exploratory/Laboratory:**
  - **Characterize NYCE T cells with respect to their expansion, persistence, trafficking, phenotype and function**
  - **Describe the incidence of immunogenicity**
  - **Evaluate the bioactivity of NYCE T cells**
  - **Follow the dynamics of the T cell repertoire**

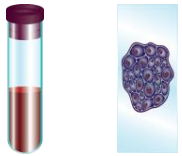
Study Schema: *NY-ESO-1-redirected CRISPR Edited T Cells (NYCE T Cells)*

ClinicalTrials.gov Identifier: NCT03399448



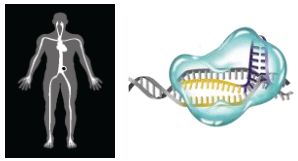
Confirmed Malignancy
- Multiple Myeloma
- Synovial Sarcoma
- Myxoid/Round Cell Liposarcoma

→



HLA-A*0201 Positive & Tumor NY-ESO-1 or LAGE-1 Positive by IHC and/or RT-PCR

→



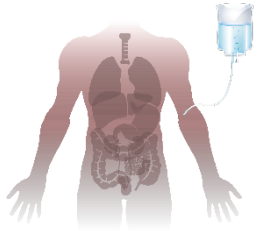
Study screening, Enrollment, Apheresis, Manufacturing

→

Multiple Myeloma, & Sarcoma Lymphodepletion
Days -5, -4, -3

300 mg/m^2 cyclophosphamide + 30mg/m^2 fludarabine

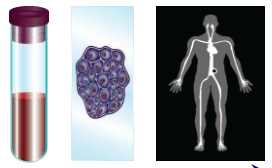
→



NYCE T cell Infusion
(1 × 10^8 total T cells/kg)
Day 0

→

Follow-up: Safety & Research



- Day 1
- Day 3
- Day 7
- Day 10
- Day 14
- Day 21
- Day 28 (Staging, Biopsy)
- Month 2
- Month 3 (Staging)
- Months 4, 5, 6
- Follow-up every 3 months until 2 years

→

Long-term Follow-up
Up to 15 years; every 6 months for up to 5 years annually thereafter for up to 10 additional years

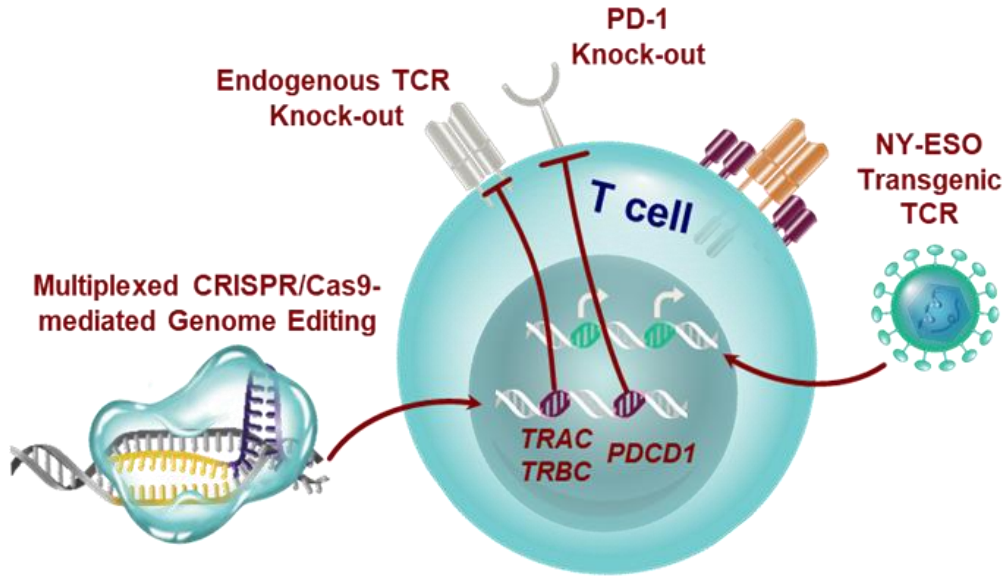
→ **Time**

Peripheral Blood *Tumor Biopsy* *Imaging* CRISPR/Cas9 *Lymphodepletion* *NYCE T cell Product*

Stadtmauer *et al, Science*, 2020

Manufacturing NYCE T cells: *Multiplexed Genomic Editing*

- **Autologous T cells**
- **Anti-CD3/CD28 bead stimulation**
- **Electroporation with ribonucleoprotein (RNP) complexes:**
  ***TRAC*/*TRBC*/*PDCD1* gRNAs + Cas9 Protein**
- **Transduction with NY-ESO-1 TCR lentiviral vector**
- **Expansion of engineered T cells**

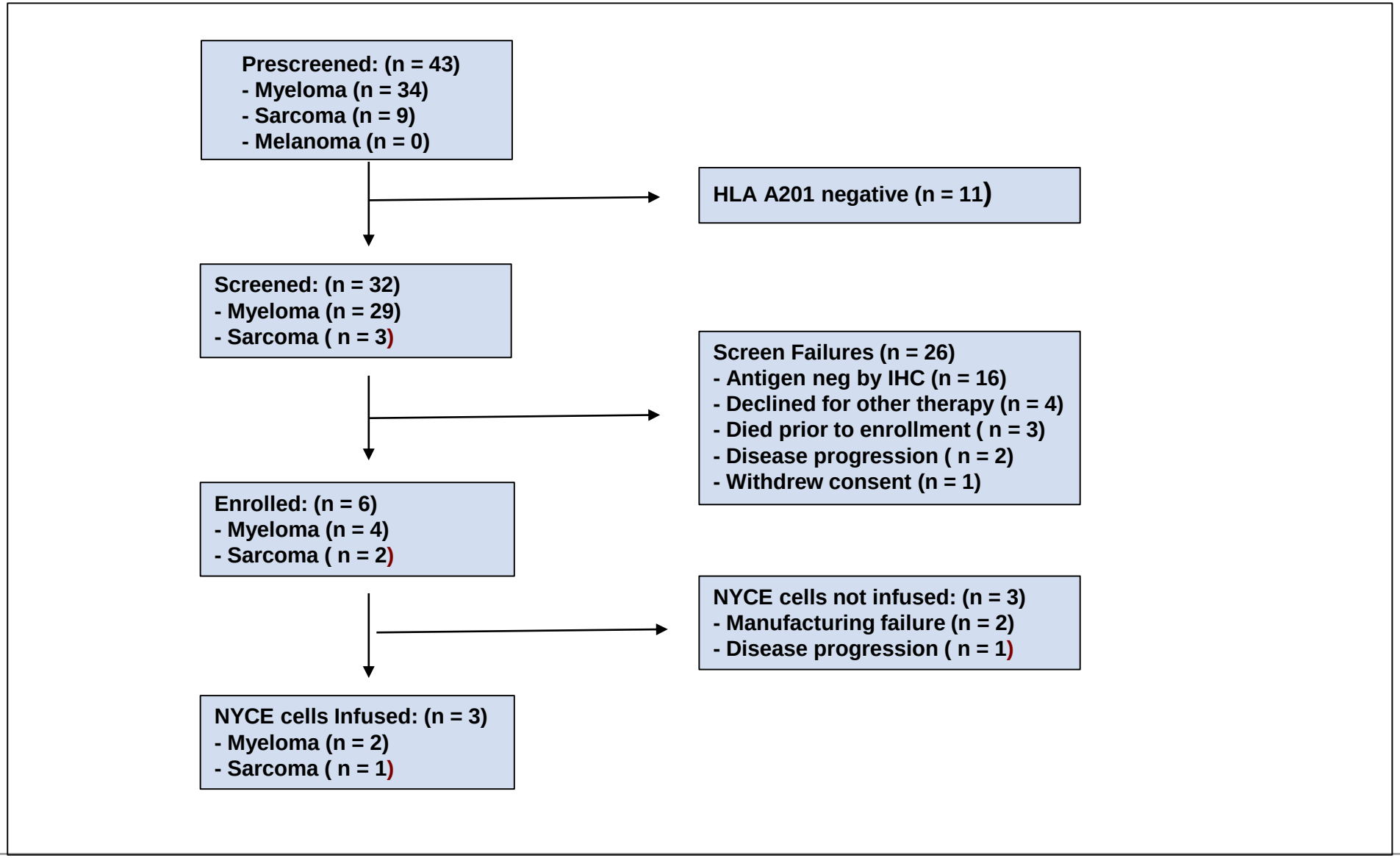


Cell Product Release Criteria

- **Viability: ≥ 70%**
- **NY-ESO TCR Transduction Efficiency (Vβ8 Flow Cytometry): ≥ 2%**
- **NY-ESO TCR Transduction Efficiency (WPRE qPCR): ≥ 0.02 - ≤ 5 Avg. copies / cell**
- **Residual Beads: ≤ 100 beads / 3 x 10^6 cells**
- **Endotoxin Content: ≤ 3.5 EU / mL**
- **Microbial Contamination: Negative**
- **Long-term Culture: No growth in the presence of IL-2 (no cell transformation)**
- **Replication Competent Lentivirus (VSV-G): < 50 Avg. copies / µg DNA**
- ***TRAC, TRBC, PDCD1* Disruption: Detectable**
- **Residual Cas9 Protein: Decreasing concentration from Day 0 to cell harvest**

Stadtmauer *et al, Science*, 2020

Study flow: Study course for participants from pretreatment consent to treatment

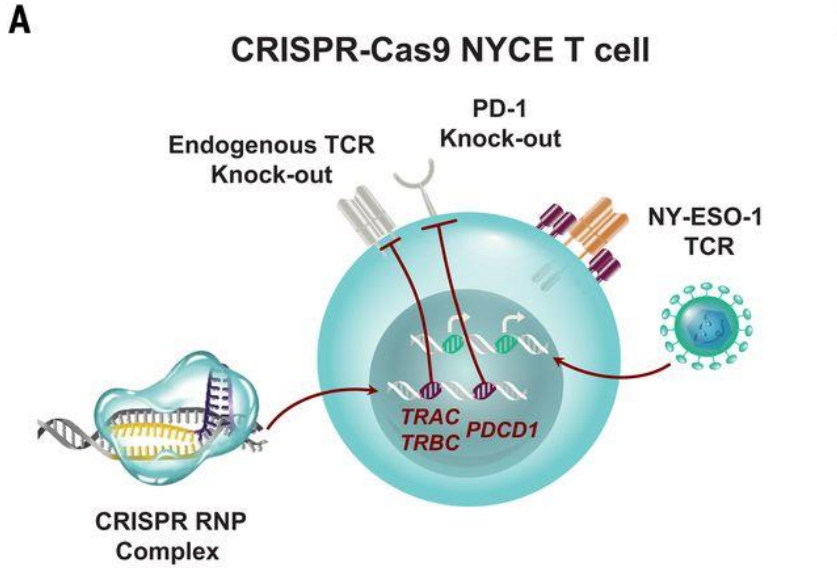


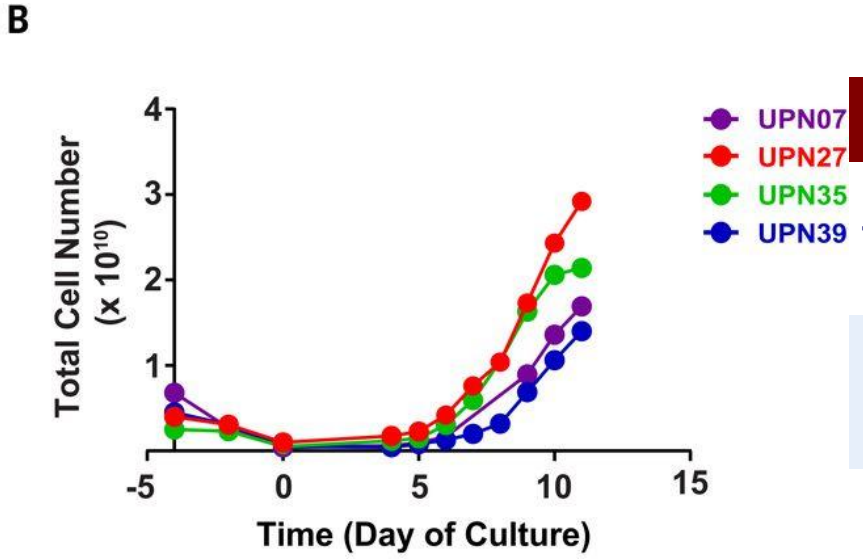
Stadtmauer *et al, Science,* 2020

Pretreatment History of Infused Subjects

Patient	Gender/ Age	Diagnosis	Clinical Sites	Prior Therapy	Prior Transplant/Surgery	LAGE-1/NY-ESO-1 (qPCR)/ NY-ESO-1 (IHC)
25416-35	Female/ 66 years	IgG kappa MM 2004	BM, lytic bone lesions	Lenalidomide, pomalidomide, bortezomib, carfilzomib, daratumumab, panabinostat (8 lines)	ASCT x 3	Pos/Pos/Neg
25416-39	Male/ 66 years	Myxoid/round cell lipo-sarcoma 2012	Abdominal /pelvic masses	Doxorubicin, ifosfamide, XRT 60-Gy, trabectedin, gemcidabine, taxol, XRT	Resection/debulking x 2, left nephrectomy and partial sigmoid resection	Not Done (ND)/ ND/Pos
25416-07	Female/ 62 years	Kappa light chain MM 2009	BM, lytic bone lesions	Lenalidomide, pomalidomide, bortezomib, carfilzomib, daratumumab, anti-CD38 immunoconjugate (6 lines)	ASCT x 2	Pos/Pos/Neg

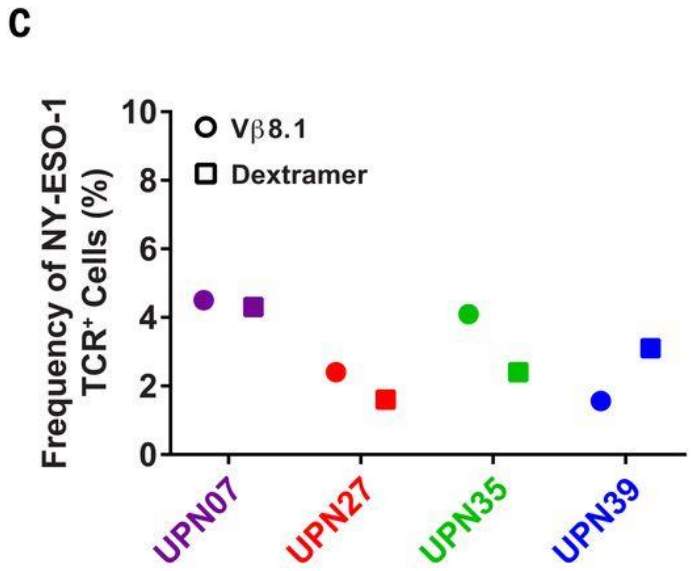
NYCE T cell Infusion Product Characteristics: Feasible

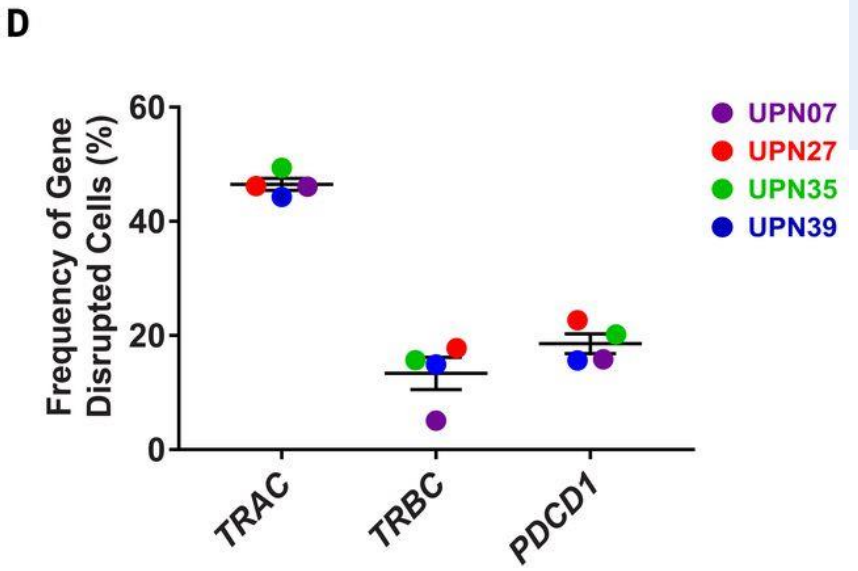




Characteristic	Mean (*n* = 4)
NY-ESO-1 TCR Transduction Efficiency	5%
TRAC Frequency of Disrupted Cells	46%
TRBC Frequency of Disrupted Cells	14%
PDCD1 Frequency of Disrupted Cells	19%
Total TCR⁺ T cells/dose*	3.59 × 10⁸
Total T cells/kg	1.00 × 10⁸
Total T cells/dose	6.70 × 10⁹

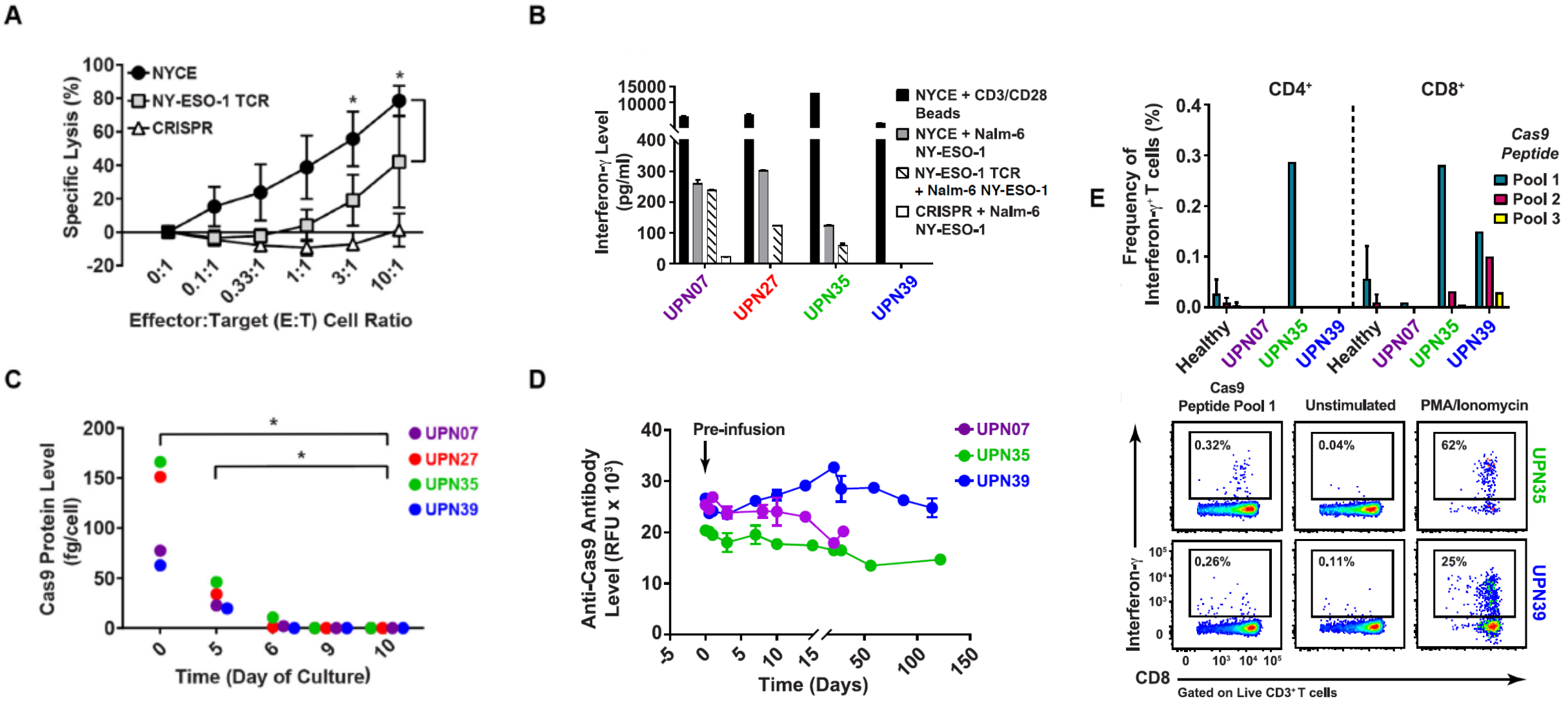
*TCR⁺ = NY-ESO-1 Transgenic TCR



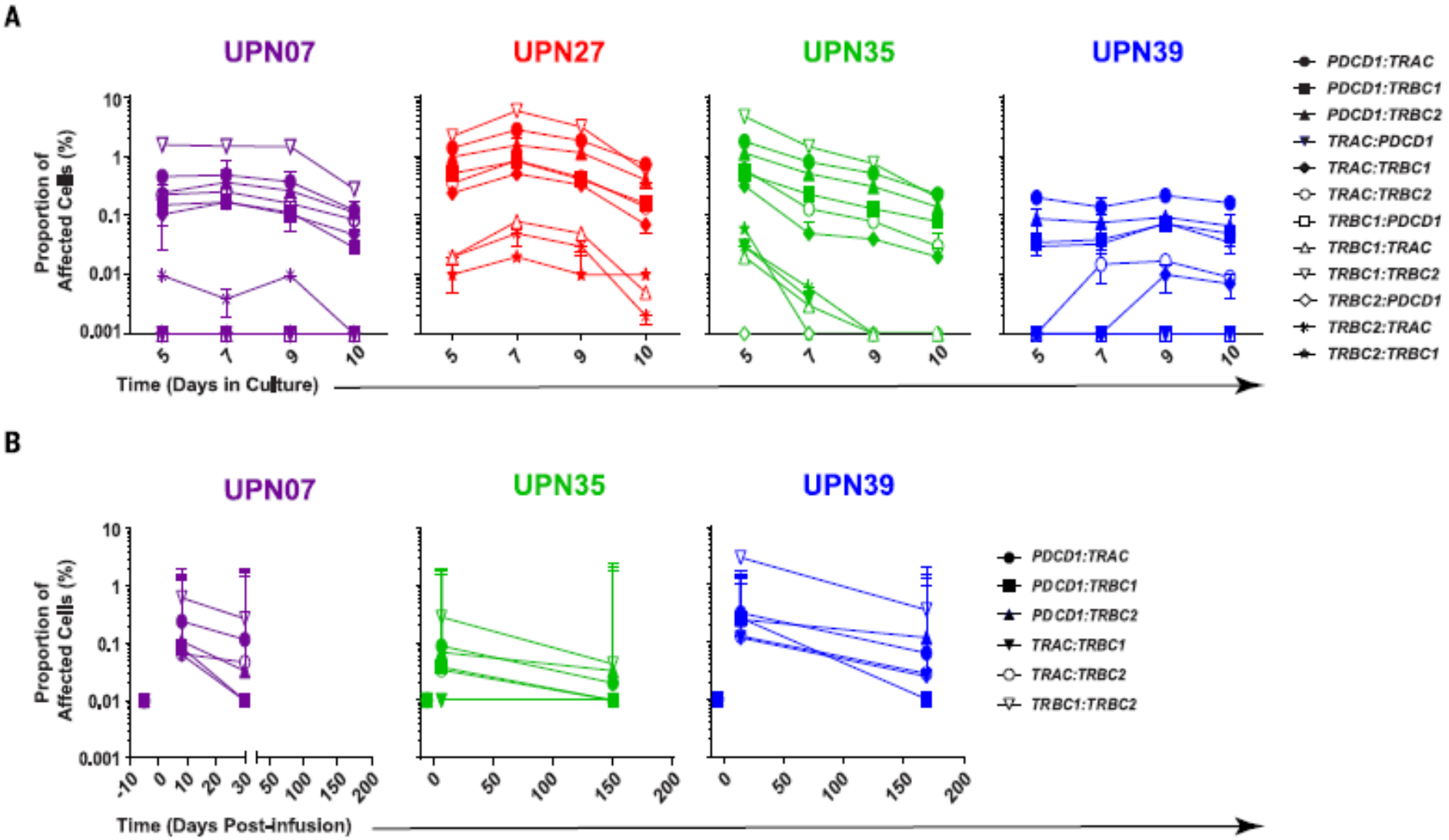


Stadtmauer *et al, Science,* 2020

NYCE T cell Infusion Product Potency, Residual Cas9 Content or Immunogenicity



Chromosomal Translocations Detected in Manufactured Products (PCR) and in vivo

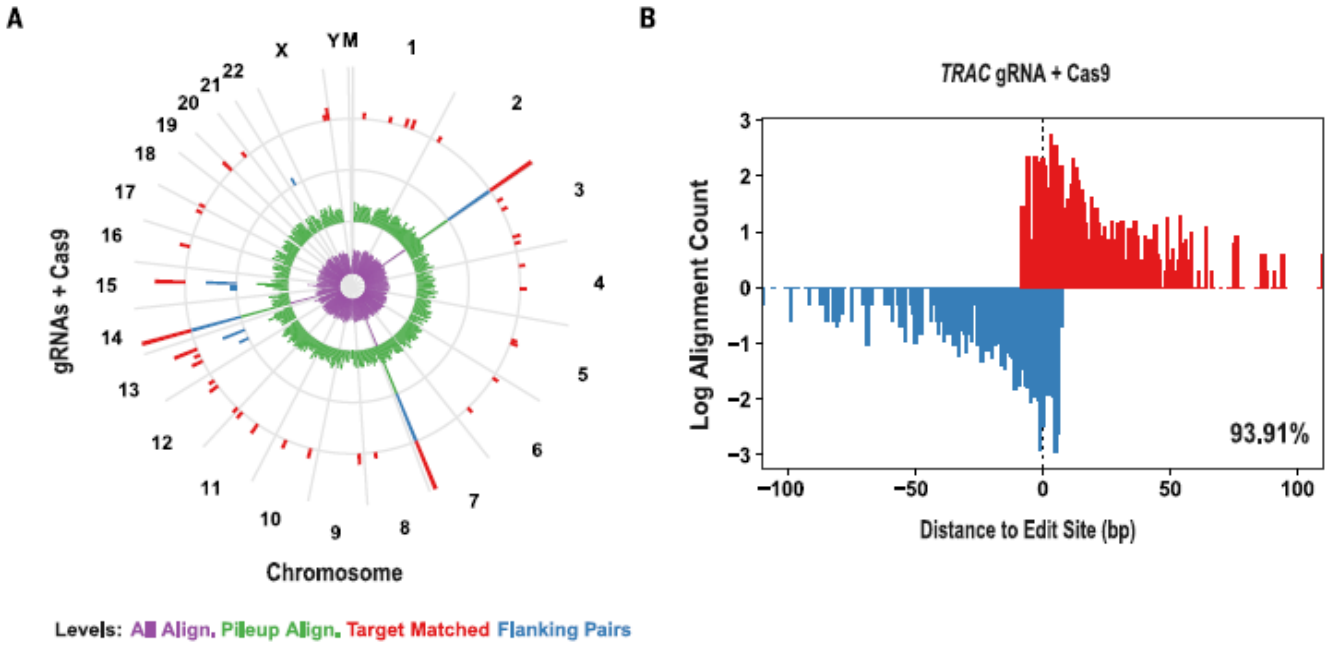


- **The edited genes are on 3 different chromosomes: 12 possible translocations detected using this assay**

- **Results show that translocations are present at day 5, but significantly decrease in frequency at harvest**

- **Suggests translocations decrease T cell fitness**

Fidelity of CRISPR-Cas9 gene editing.

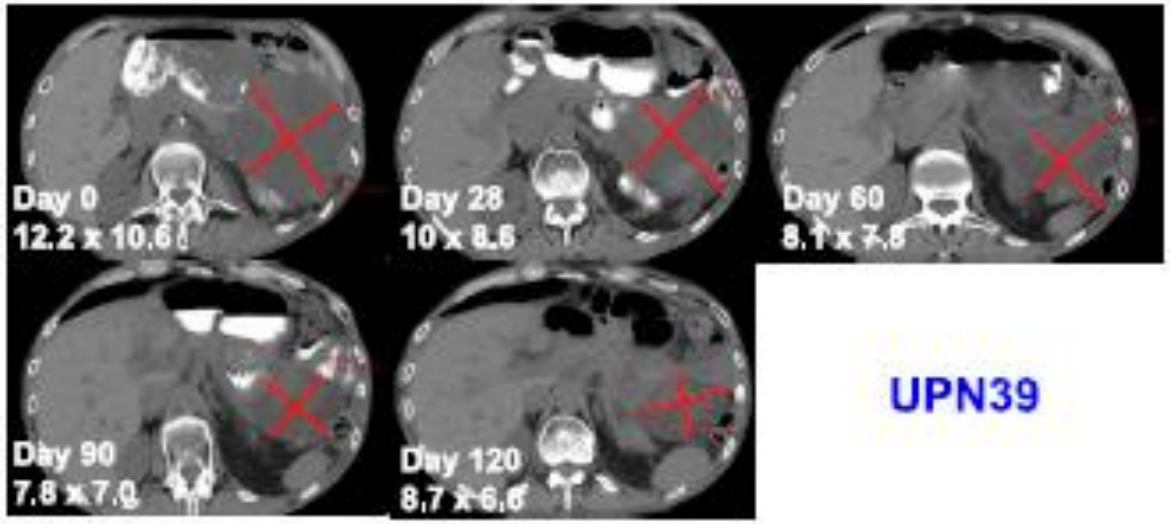


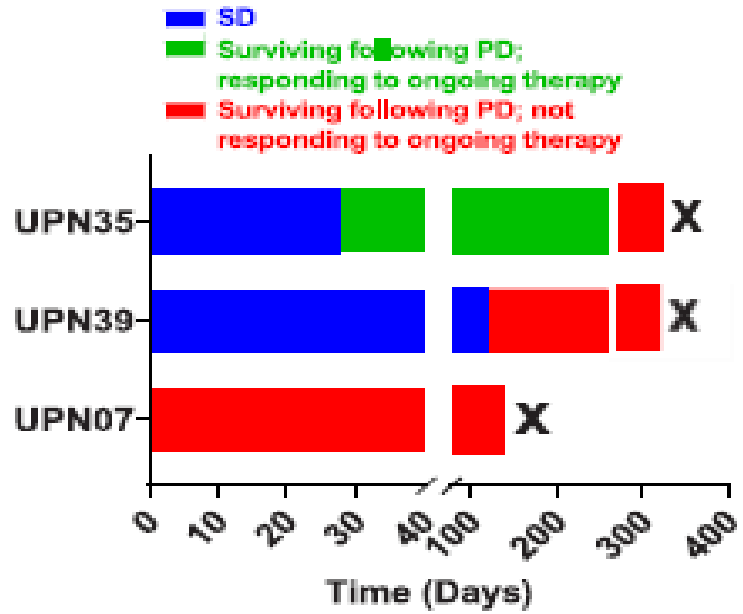
- The targeted double stranded breaks/cleavage sites are on chromosomes 2, 7, and 14.
- 93.91% is an estimate of the number of incorporations associated with the on-target site.
- Most off target insertions were at a site within the transcription unit of the specific gene and not on the allOnco cancer-associated gene list.

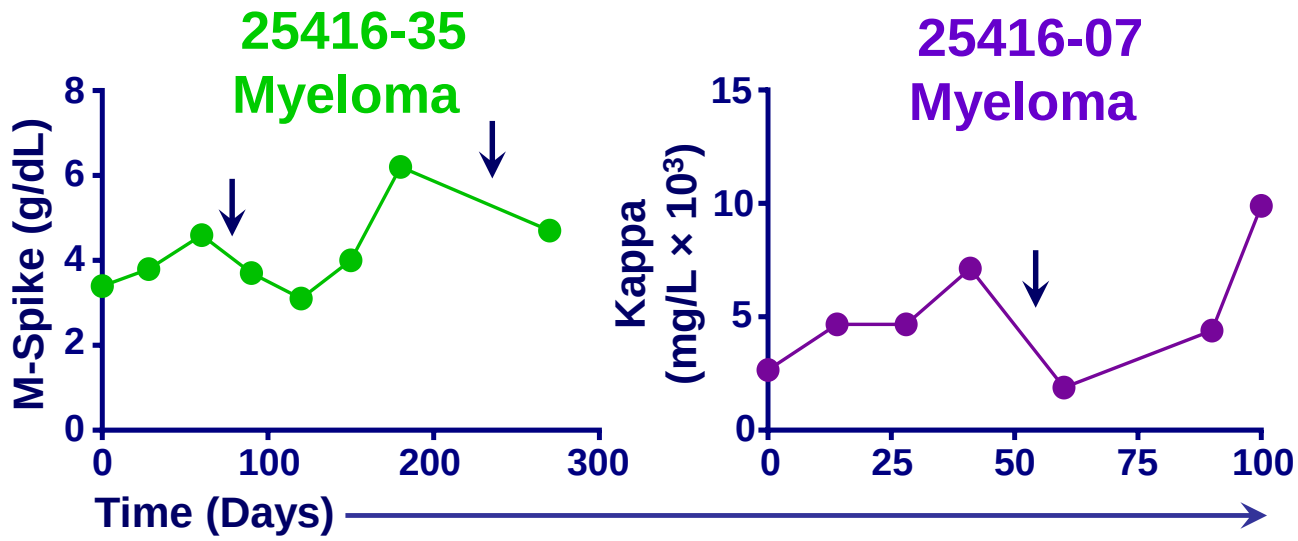
Stadtmauer *et al*, *Science*, 2020

Clinical Responses and Outcome Following NYCE T cell Infusion

- All patients received out-patient T cell infusion (at protocol-specified dose) and required no hospitalization by day 28
- No CRS or Neurotoxicity, 39 required TXN 1 U PRBC
- Best response: SD
- Subject 35 PD day 28, responding to elotuzumab, pomilidomide and dexamethasone ↓
- Subject 07 PD despite D-ACE ↓ (transient response),
- Subject 39 PD day 120, progressing on doxil but did have approximately 50% shrinkage in a large pelvic lesion







Stadtmauer *et al, Science,* 2020

Safety: *Most Adverse Events Attributed to Lymphodepleting Chemotherapy*

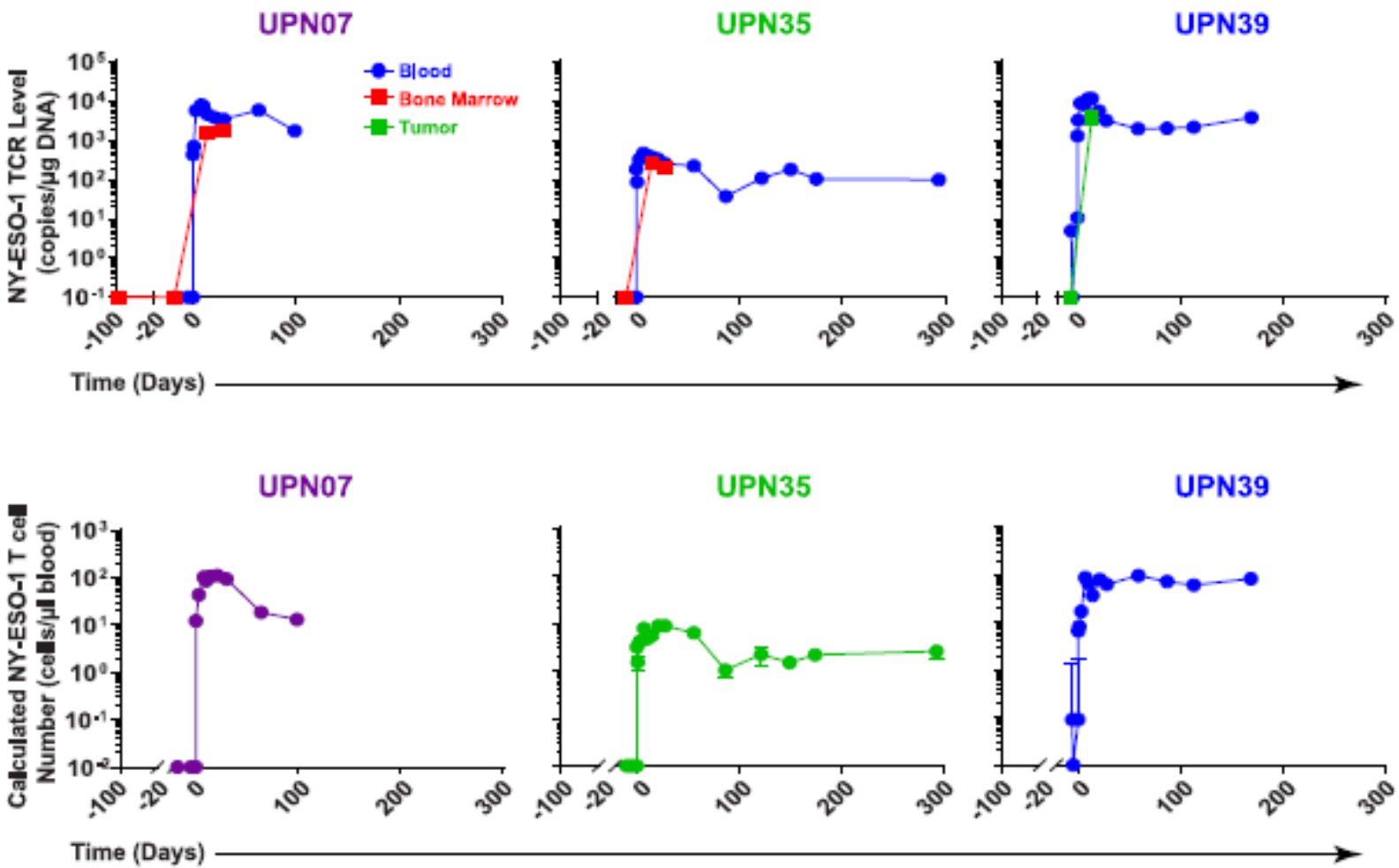
AE Category	Toxicity	All Grades	Grade 1/2	Grade 3/4
Hematologic	Anemia	2	1	1
	Leukopenia	4	-	4
	Neutropenia	4	1	3
	Thrombocytopenia	6	3	3
	Lymphopenia	1	-	1
Infection	Upper Respiratory	1	1	-
	Febrile Neutropenia	2	-	2
Electrolyte	Hypercalcemia	1	1	-
	Hyperphosphatemia	1	1	-
	Hypoalbuminemia	1	1	-
	Hypocalcemia	3	2	1
	Hypokalemia	1	1	-
	Hypomagnesemia	1	1	-
	Hyponatremia	1	1	-
	Hypophosphatemia	1	-	1
Neurologic	Dysgeusia	1	1	-
	Headache	1	1	-
	Paresthesia	2	2	-
	Syncope	1	-	1
	Pain	3	3	-
Renal	Acute kidney injury	1	1	-
	Urinary obstruction	1	-	1
Respiratory	Aspiration	1	-	1
	Nasal congestion	1	1	-
	Cough	2	2	-
Gastrointestinal	Lower GI bleed	1	1	-
	Vomiting	1	1	-
Other	Alopecia	1	1	-
	Phelbitis	1	1	-
	LE edema	1	1	-
TOTAL		50	30	20

- **No CRS**
- **No Neurological Toxicity**

- **Most AEs**
  - **Low Grade**
  - **Hematologic or Electrolyte**
  - **Related to lympho-depleting therapy or disease**
  - **Syncope, urinary obstruction, and aspiration were unrelated to T cells**

Stadtmauer *et al, Science,* 2020

Expansion, Persistence and Trafficking of NYCE T cells: *NY-ESO-1 TCR T cells (PCR)*

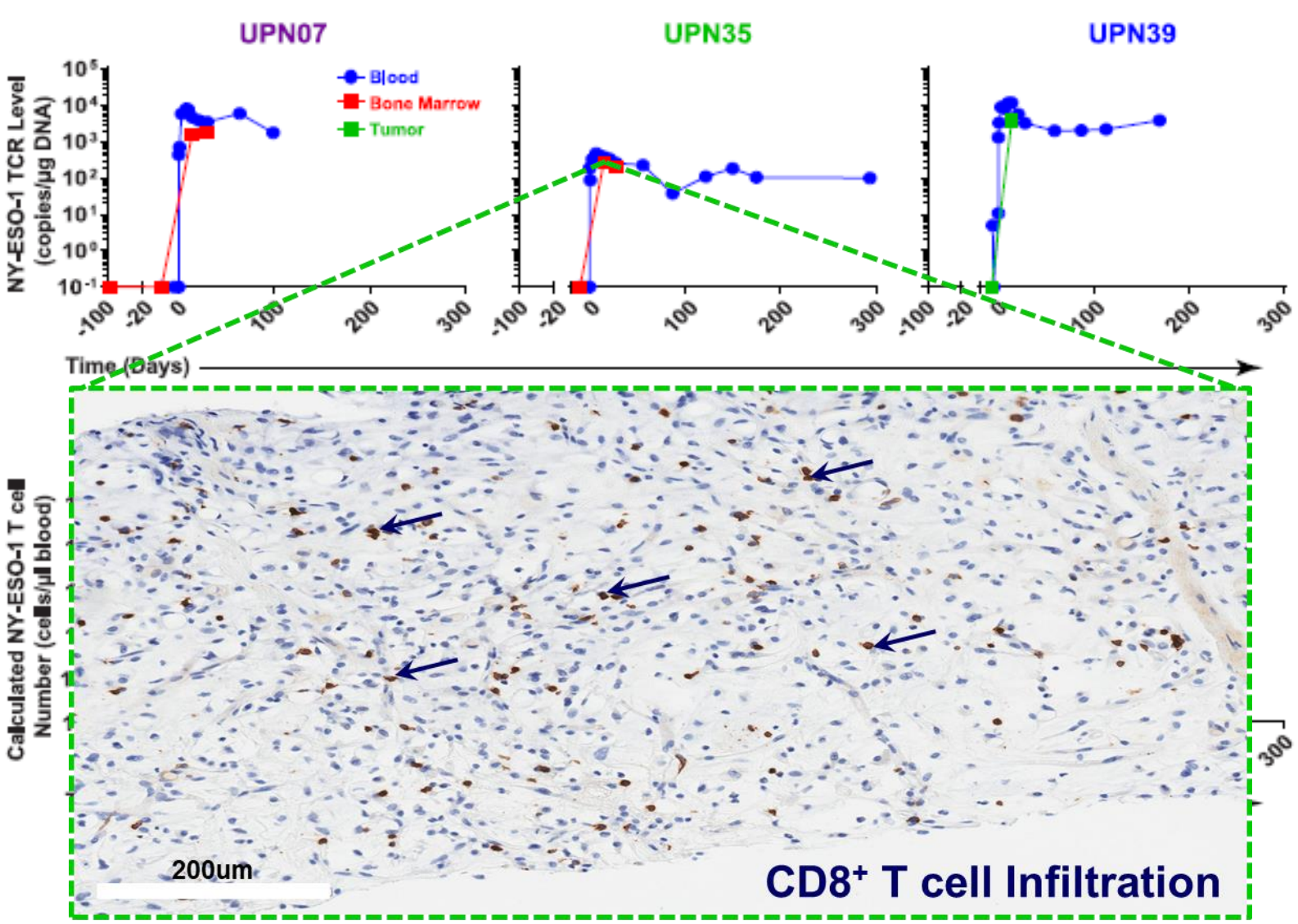


- **There is rapid expansion and stable persistence of T cells expressing the NY-ESO-1 transgenic TCR as measured by qPCR in all 3 patients**
  - **The stable PK of NY-ESO-1 expressing T cells is very different from the PK of CAR T cells which tends to decrease more quickly**

- **Clear trafficking of T cells to the tumor**
  - **The levels of T cells expressing the NY-ESO-1 TCR in bone marrow and tumor is similar to blood**

Stadtmauer *et al, Science,* 2020

Expansion, Persistence and Trafficking of NYCE T cells: *NY-ESO-1 TCR T cells (PCR)*



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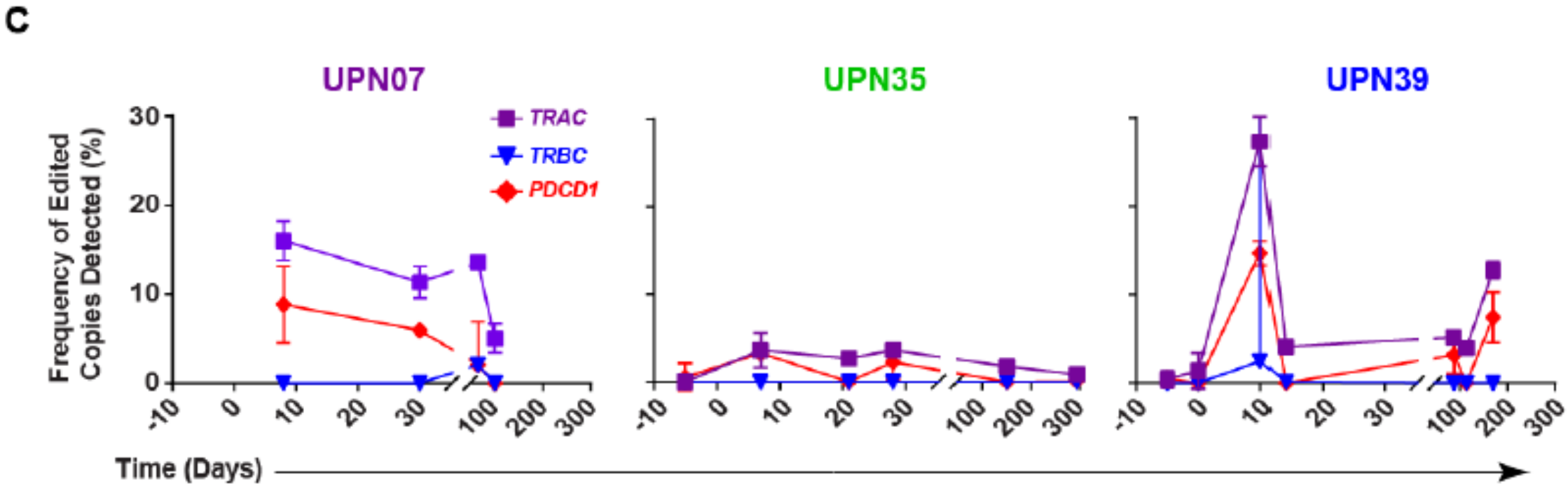
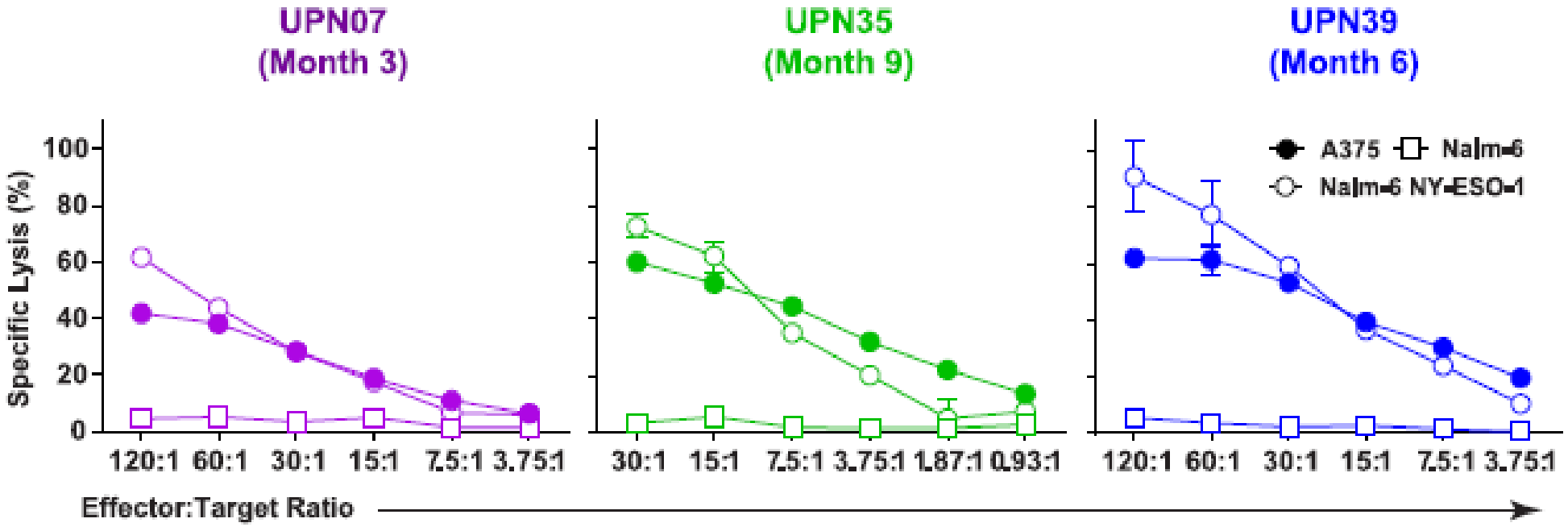
Expansion, Persistence and Trafficking of NYCE T cells: *CRISPR Edited T cells (PCR)*



- **CRISPR edited T cells expand rapidly and can persist**
  - ***TRAC (TCRα)* and *PDCD1* edited T cells have highest frequency: 5% to 10%**
  - ***TRBC (TCRβ)* edited T cells were lowest in frequency *in vitro*, and also *in vivo***
- **Subject 35 has long-term engraftment of NY-ESO-1 TCR-expressing T cells, but low frequencies of edited cells by 30 days**

Stadtmauer *et al, Science,* 2020

Cytolytic capacity of NYCE T cells preserved up to 9 months after infusion



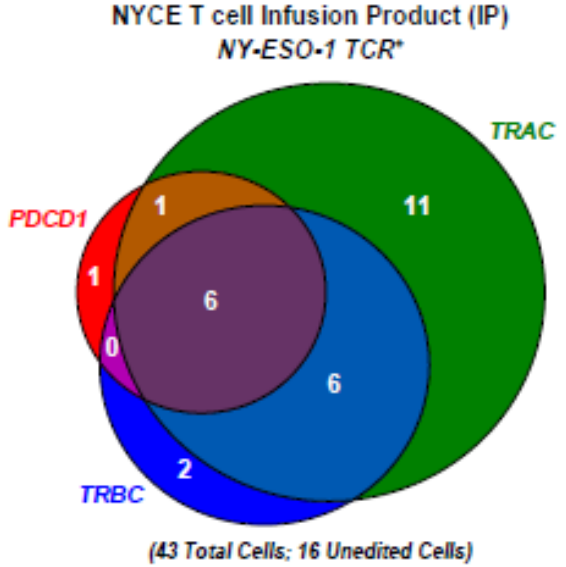
Cytolytic capacity of NY-ESO-1–specific CD8+ T cells recovered at the indicated month after infusion and expanded in vitro in the presence of NY-ESO-1 peptide and interleukin-2.

The ability of expanded effector cells to recognize antigen and elicit cytotoxicity was tested in a 4-hour 51Cr release assay incorporating Nalm-6 (B-cell ALL) NY-ESO-1+, A375 melanoma cells (NY-ESO-1+) and parental Nalm-6 (NY-ESO-1−) as control.

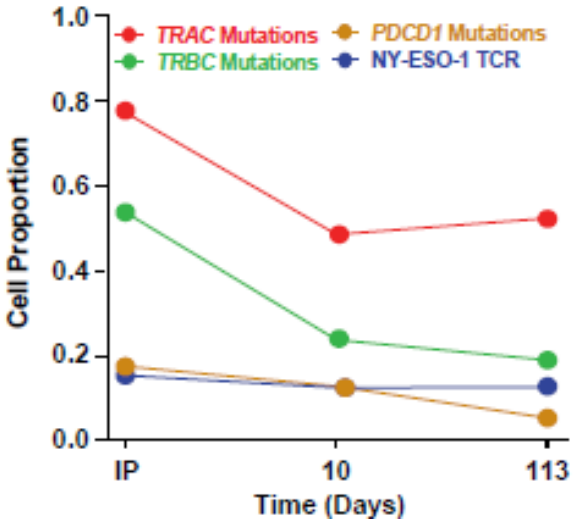
All target cell lines were HLA-A*02 positive.

Stadtmauer *et al, Science,* 2020

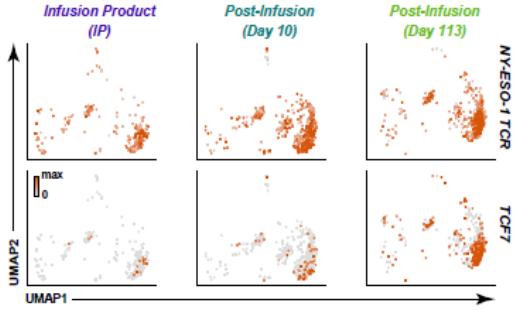
Expansion, Persistence and Trafficking of NYCE T cells: *CRISPR Edited T cells (PCR) 39*

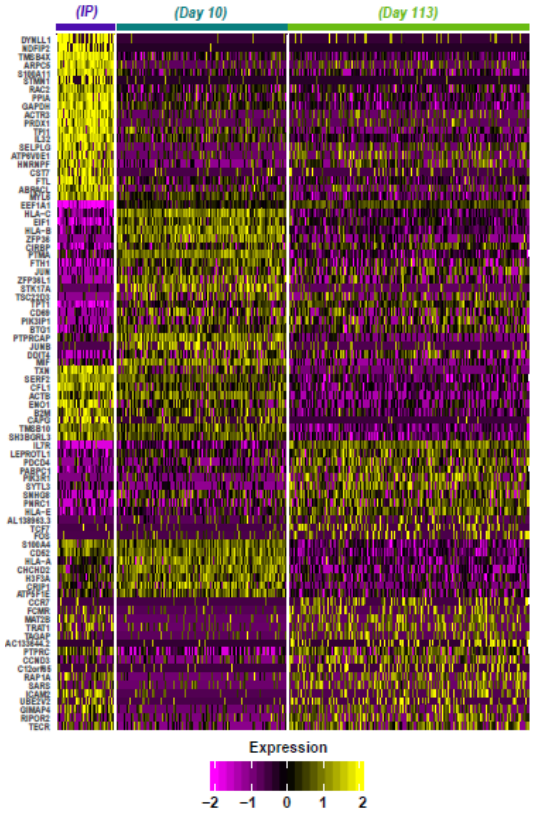


A) In the infusion product cells with all 3 mutations were found.
TRAC most common,
40% 1 mutation,
20% 2 mutations
10% 3 mutations
30% no mutations



B) Frequency of gene edited cells is stable between day 10 and 4 months after infusion



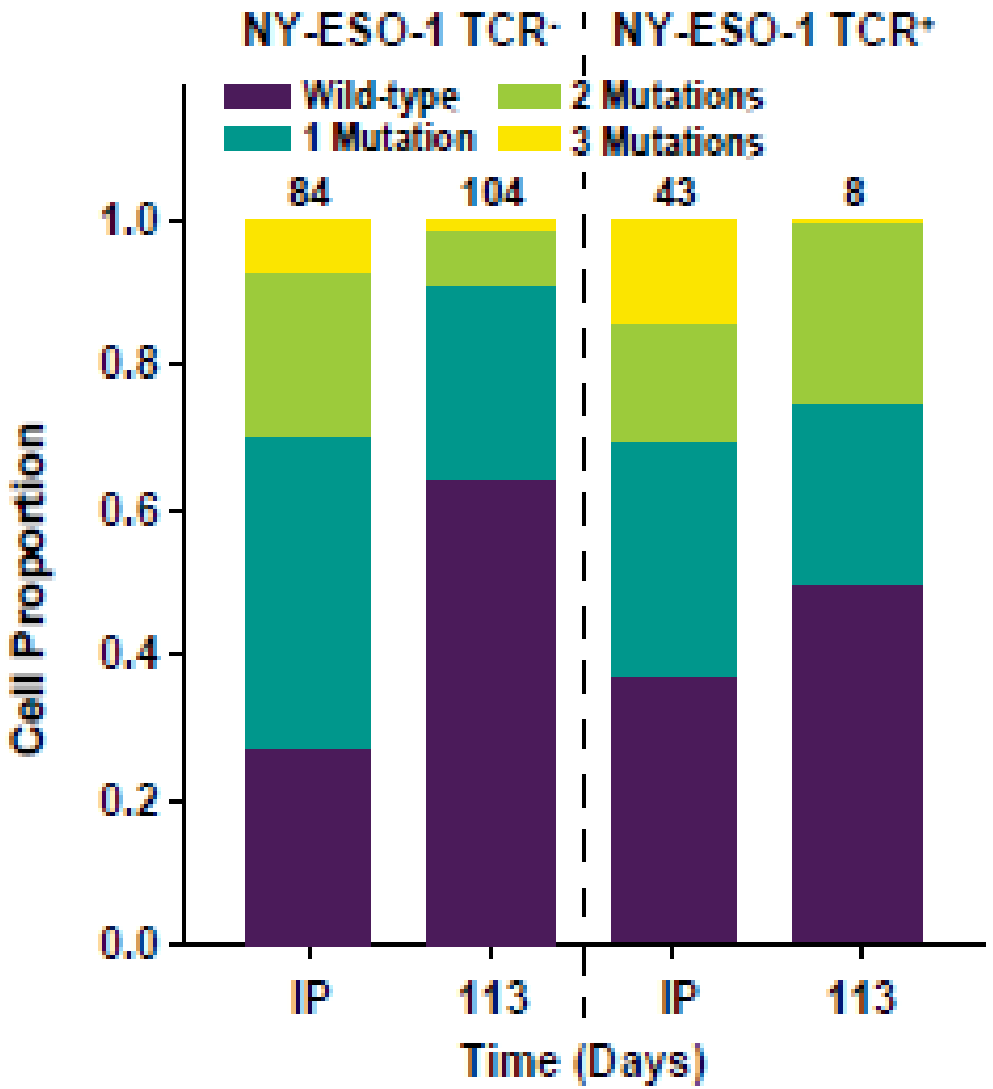


D) UMAP plots highlighting NY-ESO-1 TCR-expressing and TCF7-expressing cells sampled from each time point (infusion product, Day 0 – post-infusion, Day 113).

E) Heat map showing scaled expression of discriminative gene sets in NY-ESO-1 TCR-positive T cells over time.

The heat map and UMAP plots show increased expression of gene associated with central memory (IL7R,TCF7) over time rather than T cell exhaustion

Single-cell RNA sequencing of patient UPN39 NYCE T cell product pre- and post-infusion.



Analysis of NY-ESO-1 TCR positive (right) and –negative (left) cells without mutations (wild-type) or with single, double or triple mutations at Day 0 (NYCE T cell infusion product) and Day 113 post-NYCE T cell infusion.

A decline in the frequency of gene-edited T-cells at 4 months from levels in the infusion product is seen, but a remarkable 40% of circulating T cells at 4 months are still mutated in at least 1 of the target genes.

Stadtmauer *et al, Science,* 2020

Conclusions

- **Generation of multiplexed genetic engineering of autologous T cells expressing NY-ESO-1 TCR and CRISPR/Cas9 gene edited to eliminate endogenous TCR and PD-1 (NYCE T cells) is feasible**

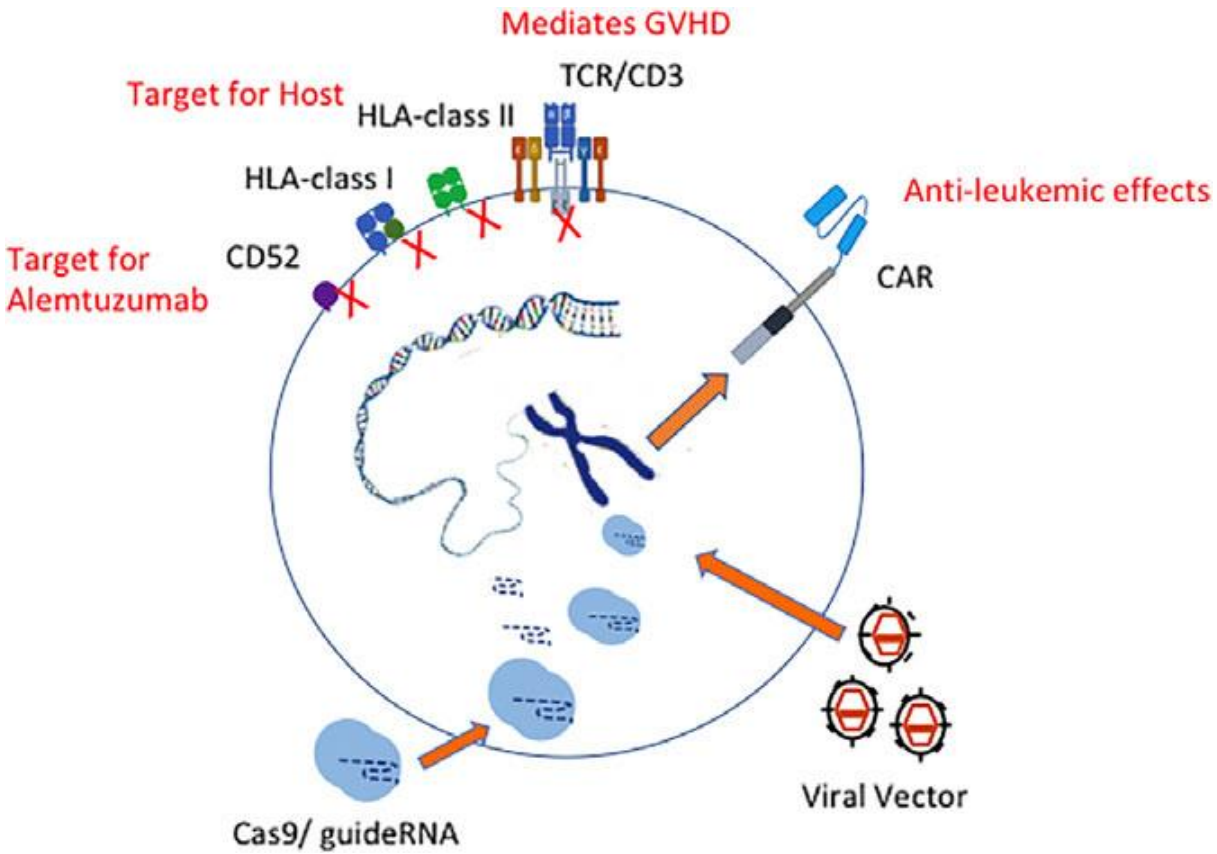
- **Three patients with advanced cancer have safely received NYCE T cells after lymphodepletion**

- **Engineered T cells expand, survive and persist long-term in patients**

- **Best overall response achieved after NYCE T cell infusion to date is stable disease**

- **So, where do we go from here?**

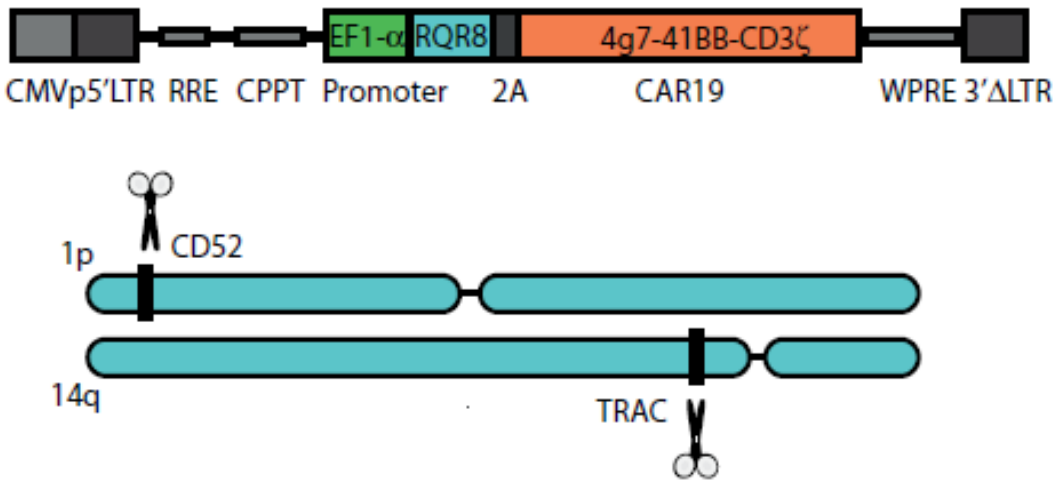
NEXT: Allogeneic CAR T Cell Therapies for Cancer



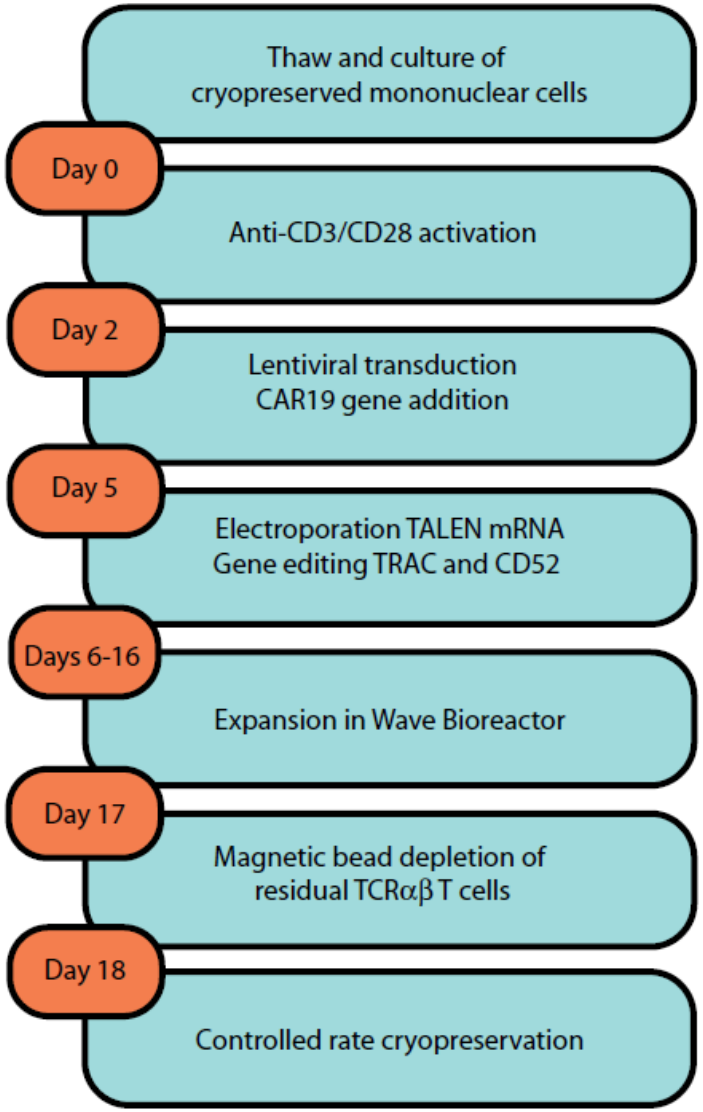
- Allows for an 'off the shelf' product for timely therapy
- Genome modified CAR T cells to prevent alloreactivity:
  - Disruption of T cell receptor
    - alpha (TRAC) or beta chains (TRBC1/2).
  - Simultaneous editing of targets to address host mediated rejection
    - (HLA class I and possibly class II molecules)
- TALEN or CRISPR Cas9 gene editing

Qasim, *Am J Hematol*. 2019;94:S50–S54.

Molecular remission of infant B-ALL after TALEN gene-edited CAR T cells

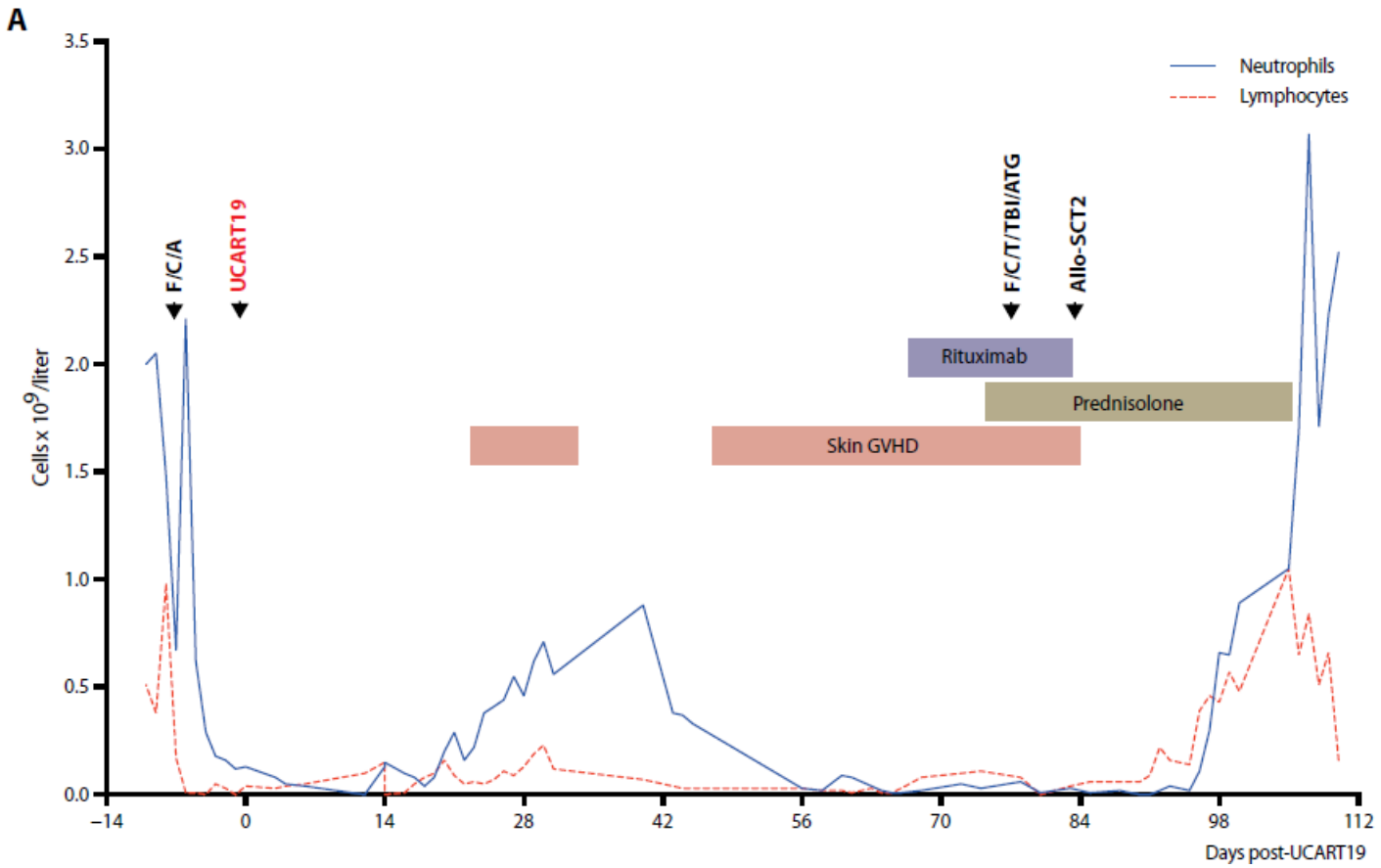


Qasim et al., generated universal CAR19 (UCART19) T cells by lentiviral transduction of HLA mis-matched donor cells and simultaneous TALEN–mediated gene editing of T cell receptor a chain and CD52 gene loci.



Qasim et al., *Sci. Transl. Med*. 2017

TALEN gene-edited CAR T cells Treatment Protocol



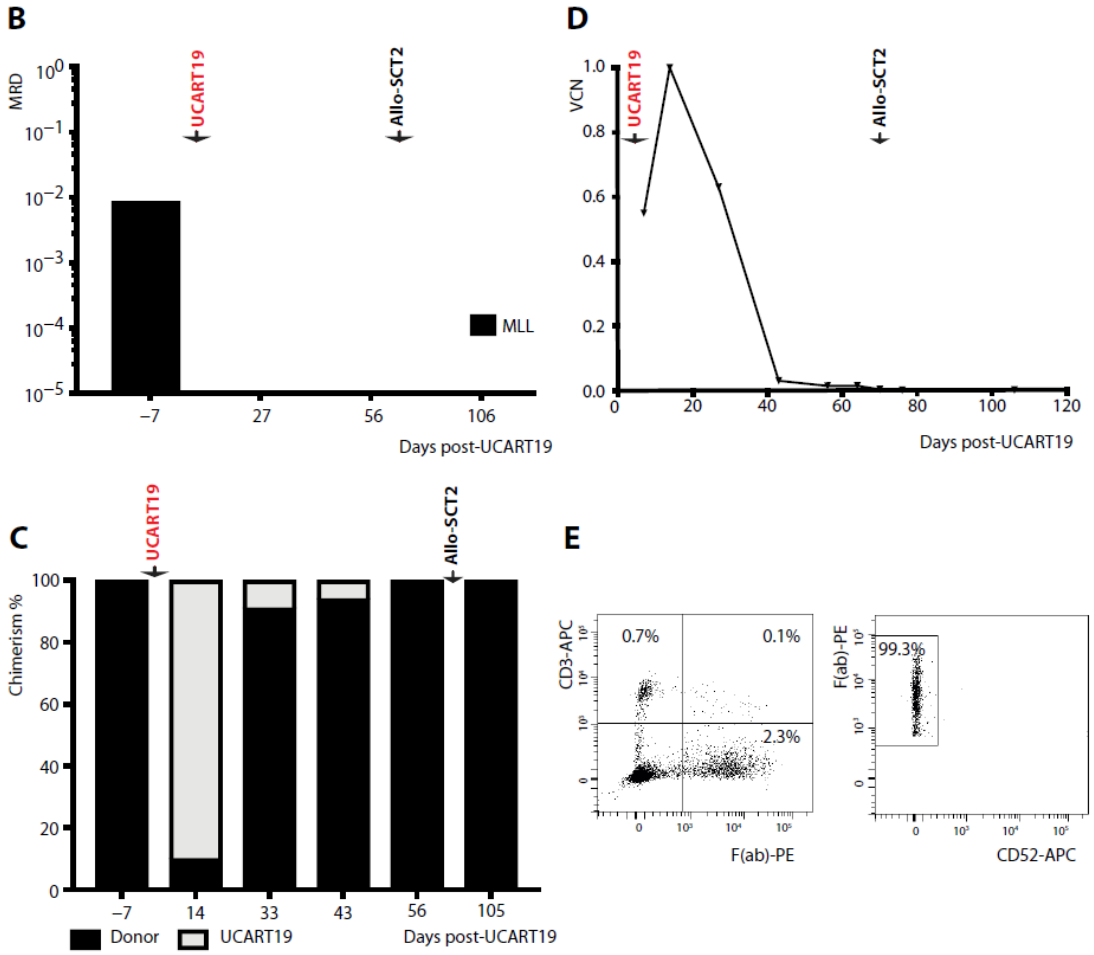
Two infants with relapsed refractory CD19+ B cell ALL despite allogeneic HSCT received lymphodepleting chemotherapy and anti-CD52 serotherapy (Alemtuzumab), followed by a single-dose infusion of UCART19 cells.

Protracted multilineage cytopenias were induced but molecular remissions were achieved within 28 days and UCART19 cells persisted until conditioning ahead of successful second allogeneic stem cell transplant.

GVHD of skin was treated with prednisolone (1 mg/kg) and four doses of rituximab (375 mg/m2).

Qasim et al., *Sci. Transl. Med*. 2017

TALEN gene-edited CAR T cells Treatment Results



(B) PCR quantification for ALL was positive after the first allo-SCT and became negative after UCART19 therapy.

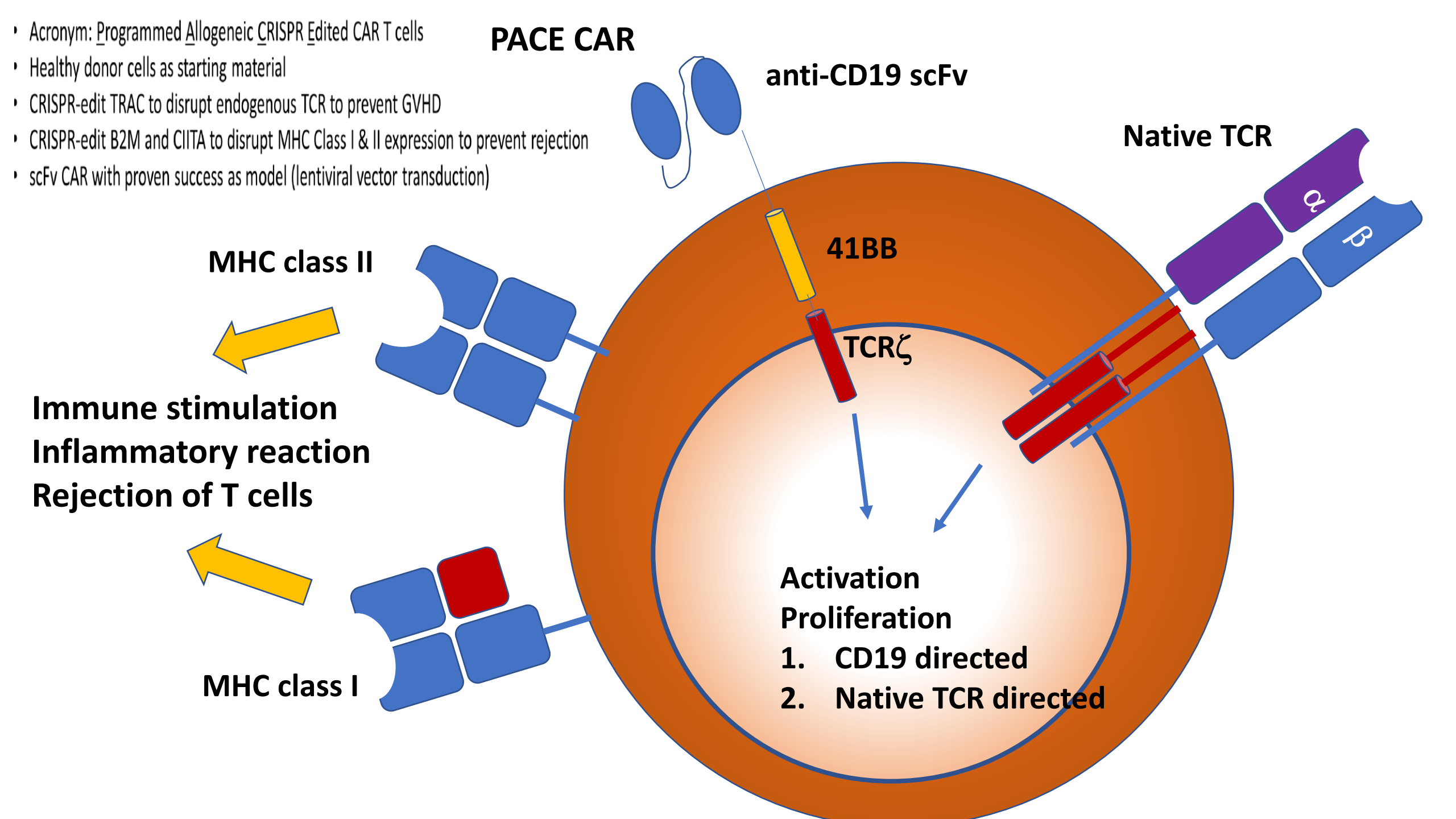
(C) Chimerism studies detected the original allo-SCT donor and UCART19 cells until the second allo-SCT.

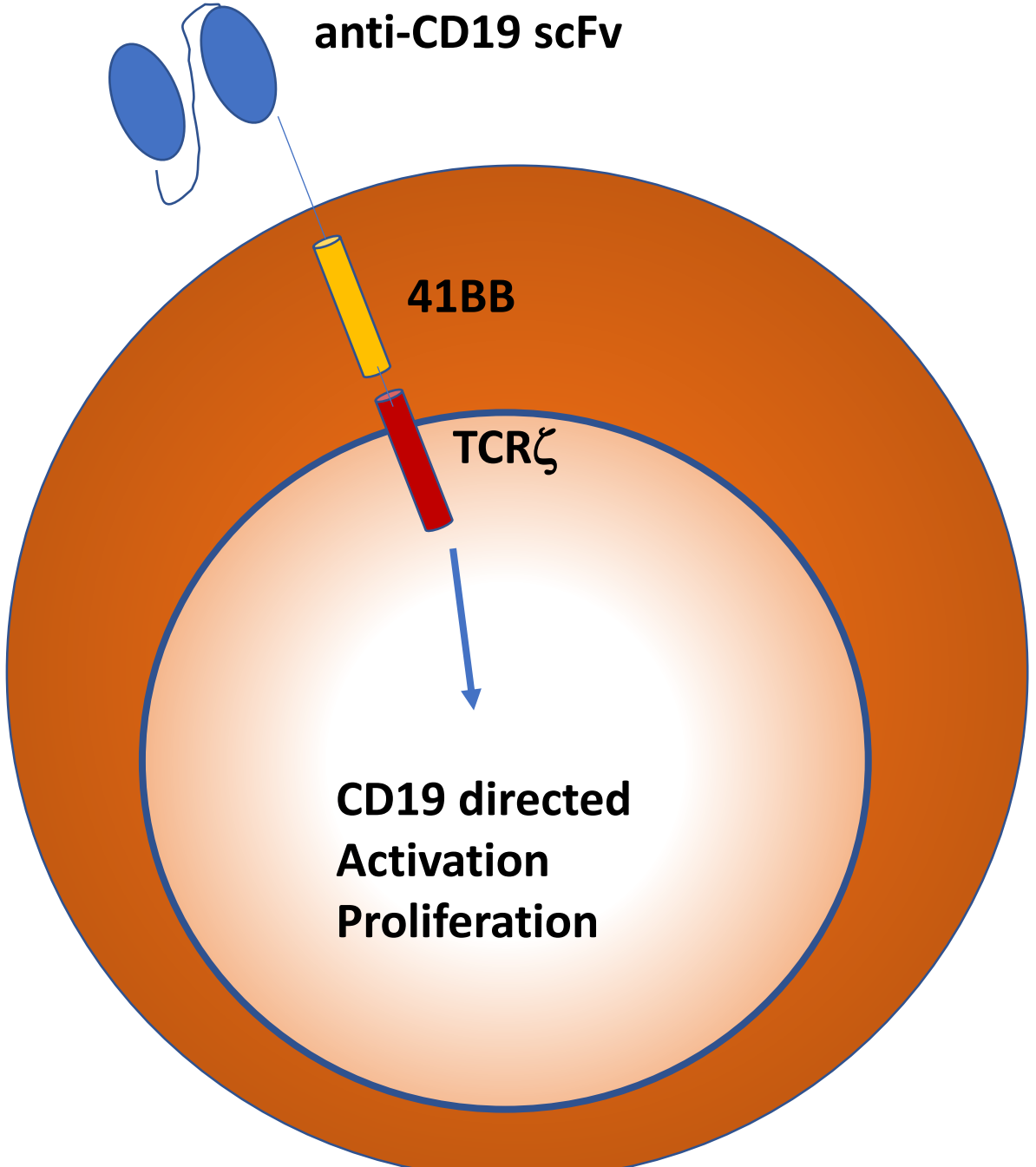
(D) UCART19 cells were detected, peaking in the third week after infusion, and persisting at low levels until conditioning ahead of second allo-SCT at 10 weeks after infusion.

(E) Almost all circulating T cells that expressed CAR19 were CD52− and CD3− on flow cytometric analysis.

Qasim et al., *Sci. Transl. Med*. 2017

- Acronym: Programmed Allogeneic CRISPR Edited CAR T cells
- Healthy donor cells as starting material
- CRISPR-edit TRAC to disrupt endogenous TCR to prevent GVHD
- CRISPR-edit B2M and CIITA to disrupt MHC Class I & II expression to prevent rejection
- scFv CAR with proven success as model (lentiviral vector transduction)

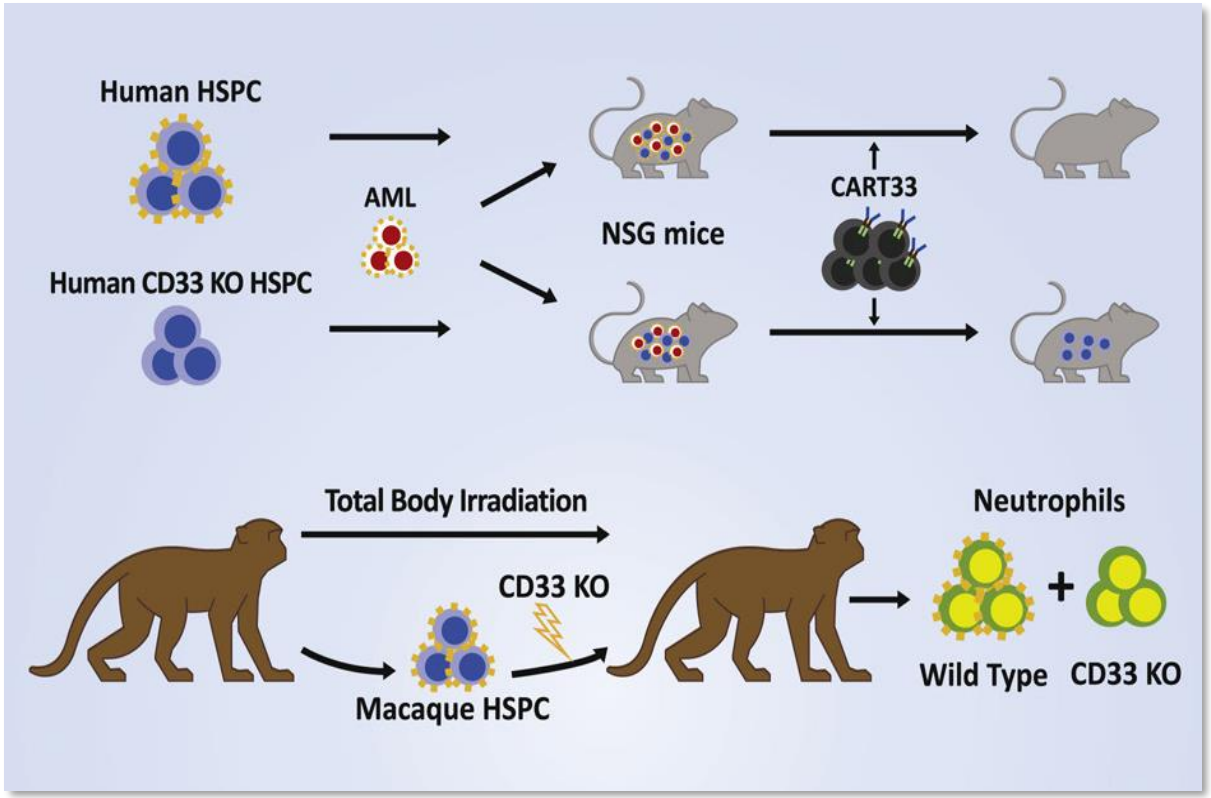



anti-CD19 scFv
41BB
TCRζ
CD19 directed
Activation
Proliferation

Genetic inactivation of CD33 in HSCs enabling CAR-T for AML

- Generated CD33-deficient human HSPCs with normal function in NSG mice
- Autologous CD33 KO HSPC in rhesus macaques provided long-term multi-lineage engraftment and normal myeloid function
- CD33 KO HSPCs were protected from CD33-targeting CAR T cells
- IND filed for ACC first-in-human study
- IMPACT: Novel strategy to target AML with CAR T cells while avoiding on-target, off-tumor toxicity

Making CD33 a tumor specific CAR target



Kim et al., *Cell*, 2018

Kenderian et al., *Leukemia*, 2015

Other Applications of CRISPR in CAR T Therapeutics

- **Production of allogeneic universal CAR T cells**
  - ZFNs and TALENs were successfully used to knock-out TCR*α* (TRAC) and TCR*β* (TRBC) to generate TCR negative CAR T cells to prevent GVHD without compromising CAR-mediated cytotoxicity

- **Disruption of inhibitory signaling molecules to overcome T cell exhaustion**
  - Disruption of multiple inhibitory factors is expected to improve the potency of CAR T cells.

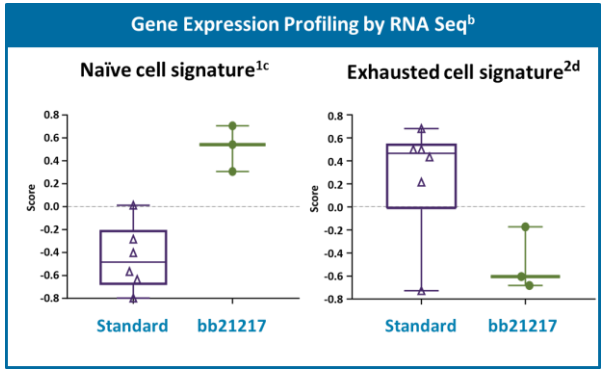
- **Reduction of cytokine release from CAR T**
  - Blocking relevant cytokines signaling is a strategy to ameliorate toxicity and CRISPR/Cas9 can effectively knock-out related molecules. CRISPR/Cas9 mediated knock-out of GM-CSF and showed GM-CSF-negative CAR T cells produced less GM-CSF without weakening antitumor activity.

- **Overcoming barriers to CAR T therapy**
  - A novel approach to circumvent the problem with potent anti-CD33 CAR T cells followed by infusions of CRISPR/Cas9-modified CD33-knockout normal HSCs, thus allowing persistent antigen-specific cytotoxicity along with reconstitution of hematopoiesis.
  - Targeted disruption of the CD7 gene using CRISPR/Cas9 prior to CAR expression minimized fratricide in T cells and allowed the expansion of the CD7-knock-out anti-CD7 CAR T cells with robust antitumor activity in preclinical models.

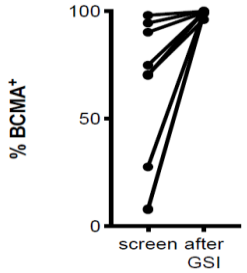
Li et al, *Briefings in Functional Genomics*, 2020

How to improve clinical outcomes?

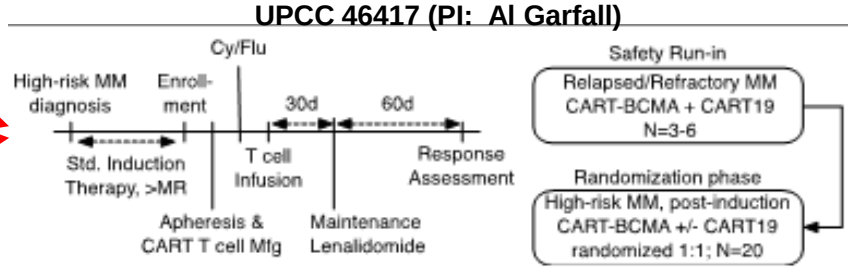
- Optimize CAR T product?
  - Dual epitope or dual antigen binding
  - Novel costimulatory domains
  - Transposon-based
  - Suicide genes/safety switches
  - Gene editing (e.g. PD-1 knockdown, allogeneic CARTs)
- Optimize manufacturing?
  - Defined CD4:CD8 ratios? Start with Tn or Tcm? PI3K inhibitors?
- Optimize target expression?
  - Gamma-secretase inhibitors for BCMA?
- Optimize infusion schedule?
  - Serial infusions? Retreatment at progression?
- Patient selection?
  - Only high expressors? Earlier lines of therapy? High-risk?
- Lymphodepletion?
  - Is Cy/Flu the best?
- Rational combinations?
  - Checkpoint inhibitors? IMiDs? Other CAR T cells?



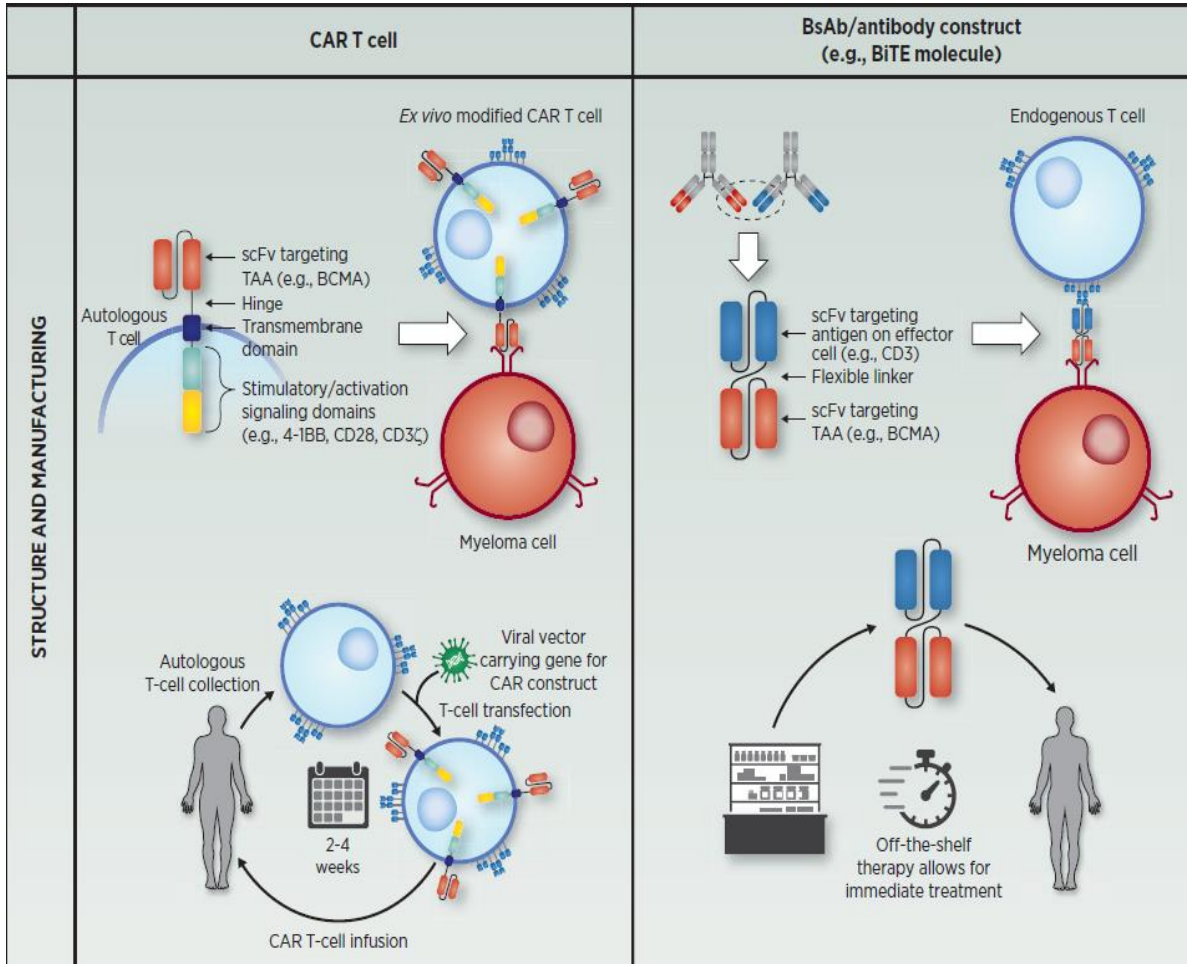
Berdeja et al, ASH 2019, #927



Cowan et al, ASH 2019, #204



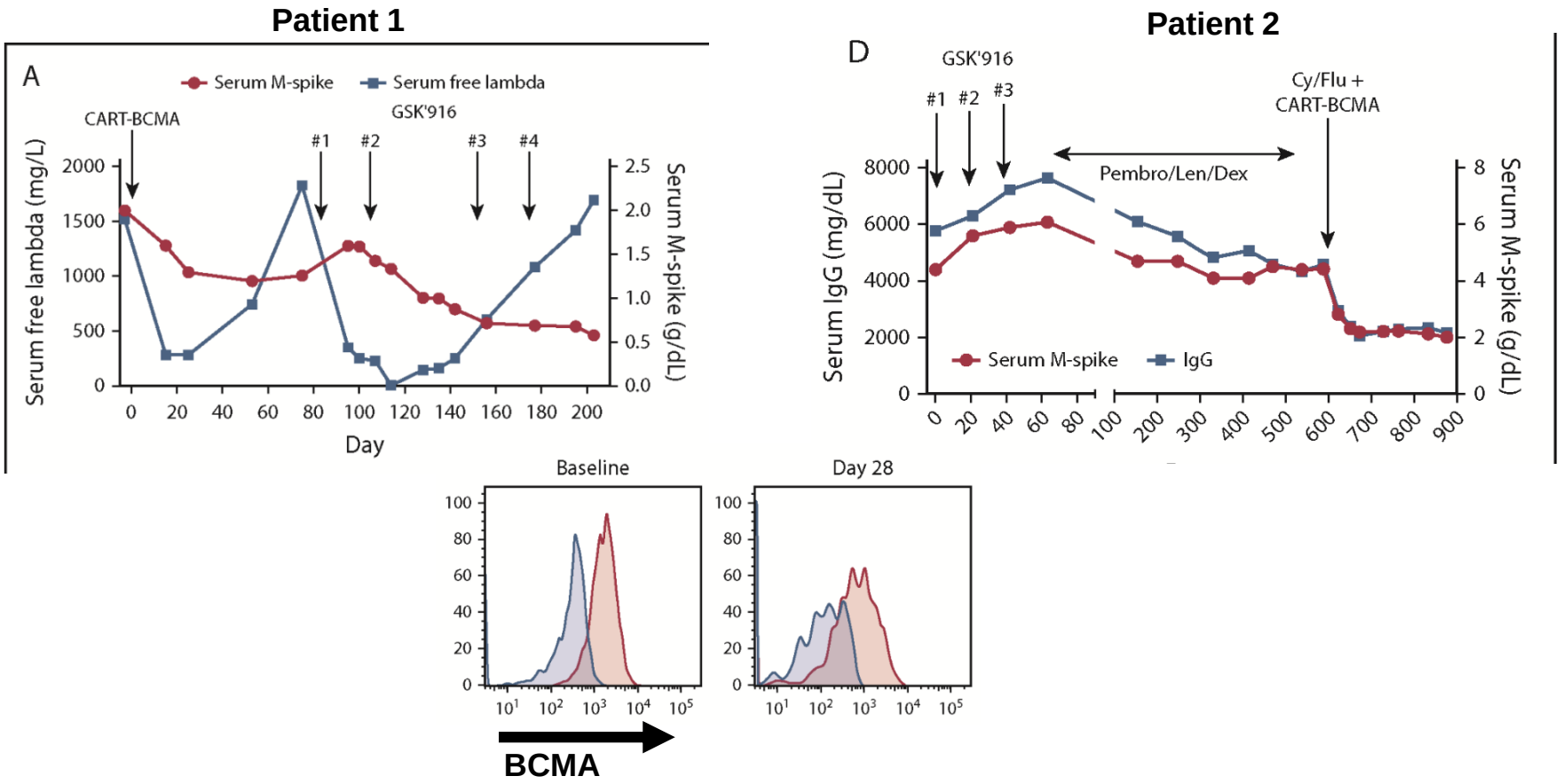
CAR T cells vs. Bispecific Antibodies (BsAbs)



	ADCs	CARTs	Bispecific Abs/BiTEs
Off-the-shelf	Yes	Not yet	Yes
Easy	++++	+	++
Repeated dosing needed	Yes	No	Yes
Depends on T cell “fitness”	No	Yes	Yes
Toxicities	IRR, Toxin-dependent	CRS, neuro	CRS, neuro
Toxicity duration	Ongoing	~7-21 days	Ongoing
Durable	Yes	Yes	Yes

Cohen et al, Clin Can Res 2019

Serial treatment with BCMA-targeted therapies



Cohen et al, Blood Advances 2019

What's Happening 2020 for Engineered T cells for Myeloma?

- **BCMA CAR registration trials in rel/ref MM**
  - Not perfect, still lots of relapses within 1 year, but remarkable responses in R/R MM without other options
  - Ongoing ph 1/2 for next-gen CAR products

- **CAR T cells against CD38, SLAMF7 (CS1), GPRC5D, NY-ESO-1**
  - These are all reasonable targets, but much more limited experience

- **BCMA CAR trials for less-heavily treated patients**
  - 1-3 priors
  - Post-induction in hi risk
    - CART-BCMA +/- CART-19 (PI: Al Garfall)
  - Post-autoSCT
    - BMT CTN ASCT + CAR T in High Risk or Poor Response

- **BCMA CAR combo trials**
  - Other CAR T cells, IMiDs, checkpoint inhibitors

- **Gene-edited T cells**
  - "Off-the-shelf" allogeneic CAR T cells
  - PD-1 deficient, endogenous TCR edited T cells (*Science* 2020)

Acknowledgements

- We thank the patients and their caregivers for taking part in these trials

- We thank the investigators and their teams at the University of Pennsylvania who participated in this work

- This study was funded by Tmunity Therapeutics and the Parker Institute for Cancer Immunotherapy

Penn Medicine
Center for Cellular Immunotherapies

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Questions?