Adoptive Cell Therapies: Keeping Pace With New and Emerging Therapies

Patricia Mangan, RN, MSN, APRN, BC
Edward Stadtmauer, MD
Abramson Cancer Center
University of Pennsylvania

Learning Objectives

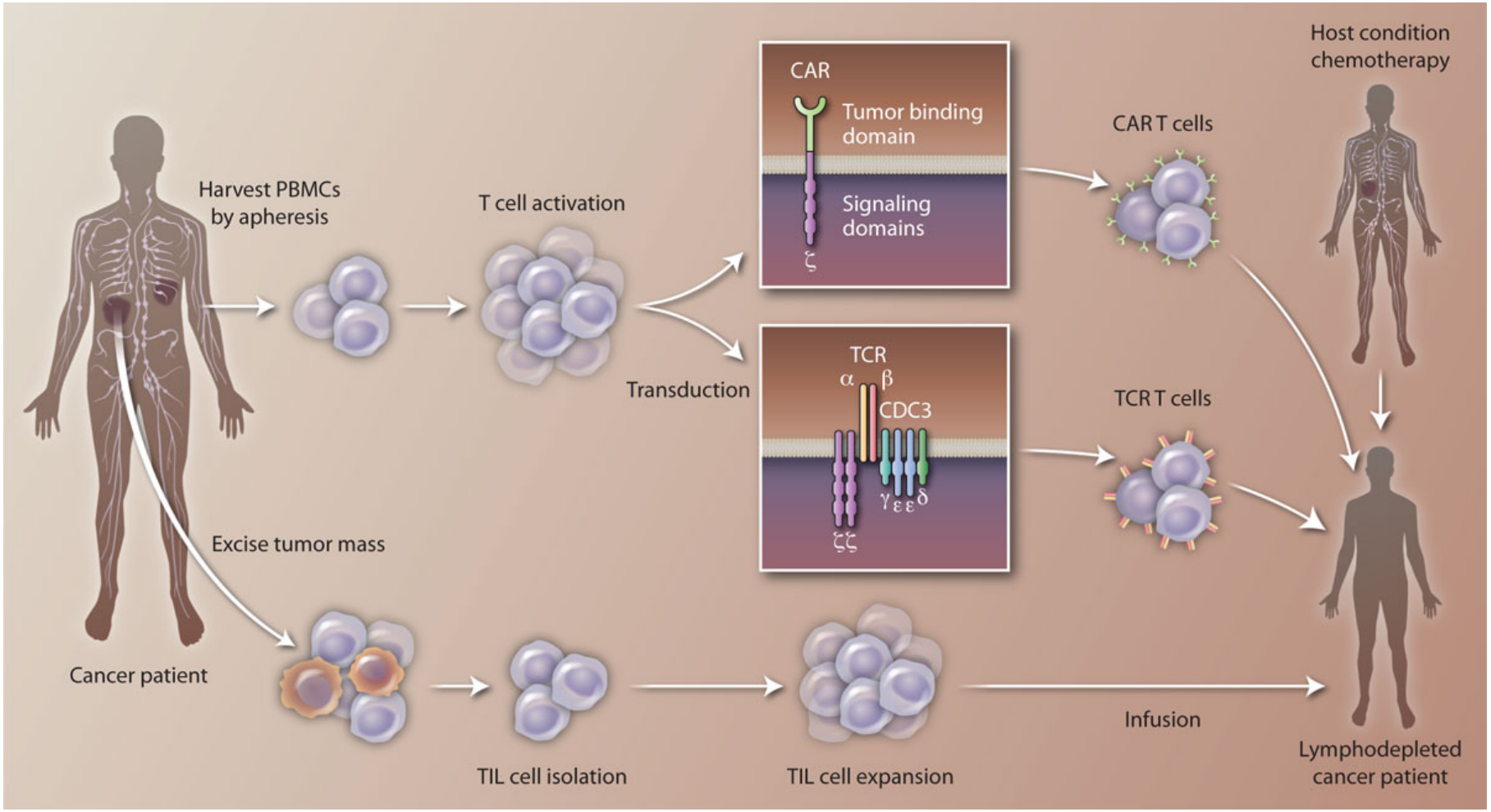
1. Review the approved indications for use of chimeric antigen receptor (CAR) T-cell therapy and studies in hematologic malignancies
2. Gain understanding of the CAR T-cell process
3. Understand the strategies for monitoring and managing emerging toxicities in patients receiving CAR T-cell therapy
4. Describe some of the future directions in the use of this therapy

Adoptive Cellular Therapy: Rationale

- Overcomes limitations of chemotherapy

- Combines advantages of:
  - Antibody therapy (specificity)
  - Cellular therapy (amplified response)
  - Vaccine therapy (memory activity)

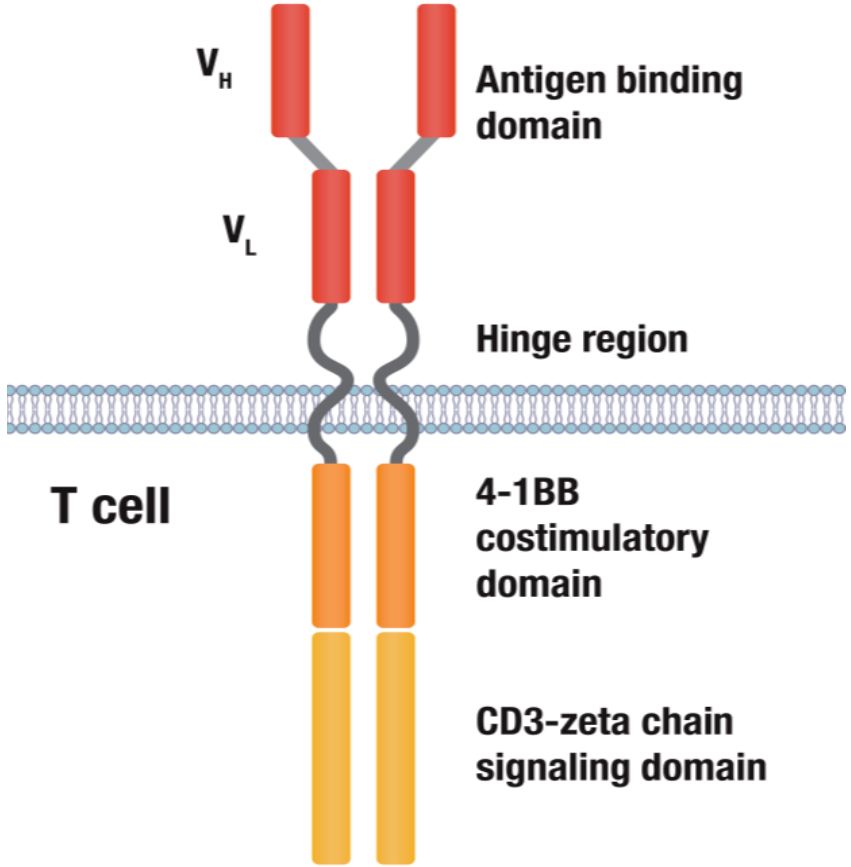
Adoptive T cell therapy (three major approaches)



June et al *Sci Trans Med 2015*

Anatomy of a Chimeric Antigen Receptor

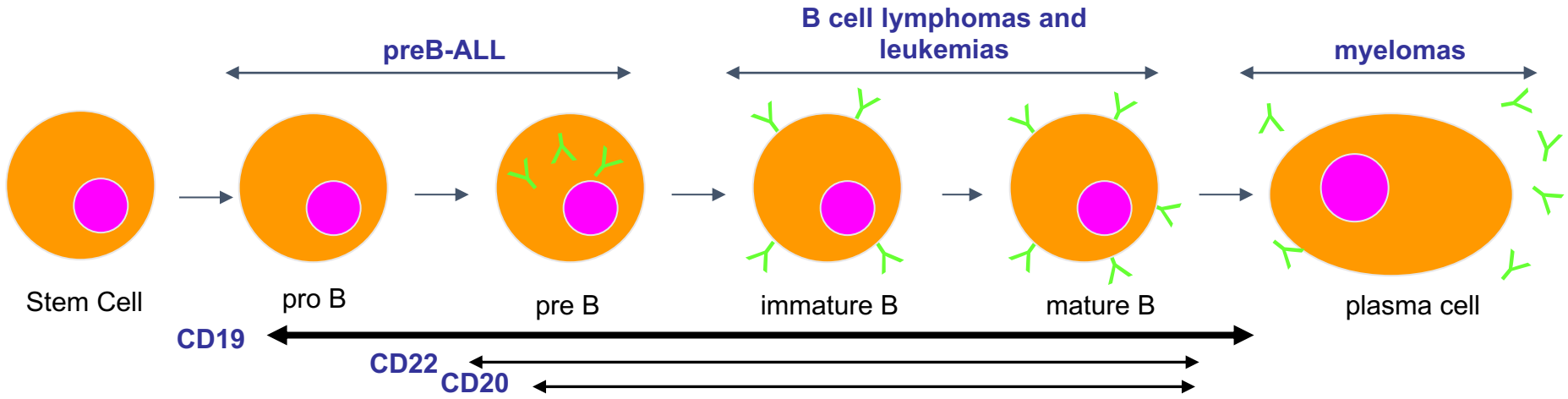
- Gene transfer technology is used to stably express CARs on T cells, conferring novel antigen specificity
- CARs combine antigen recognition domain (Anti-CD19, BCMA,CD38, CS1) with intracellular signaling domain
- Intracellular signaling domain:
  - Same functionality as endogenous T cells
  - Co-stimulatory endodomain mediates potent anti-tumor effects & promotes persistence (4-1BB, CD 28)



1. Milone, et al. Mol Ther 2009. 2. Carpenito, et al. PNAS 2009

CD19: An ideal tumor target

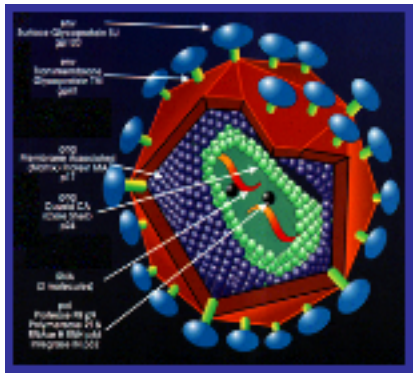
- CD19 is expressed on surface of most B cell malignancies
- CD19 expression is restricted to B cells and their precursors
- CD19 is not expressed on pluripotent bone marrow stem cells
- On target expected SE is B cell aplasia



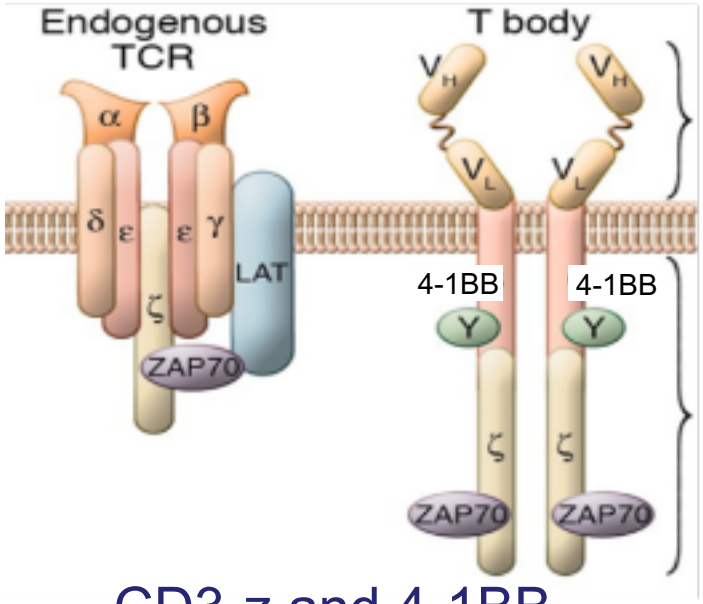
1. Scheuermann RH, et al. *Leuk Lymphoma*. 1995;18:385-397
Image adapted from Janeway CA, Travers P, Walport M, et al. *Immunobiology*. 5th ed. New York, NY: Garland Science; 2001:221-293; Scheuermann RH, et al. *Leuk Lymphoma*. 1995;18:385-397; and Feldman M, Marini JC. Cell cooperation in the antibody response. In: Roitt I, Brostoff J, Male D, eds. *Immunology*. 6th ed. Maryland Heights, Missouri: Mosby;2001:131-146.

CAR for B Cell Malignancy:

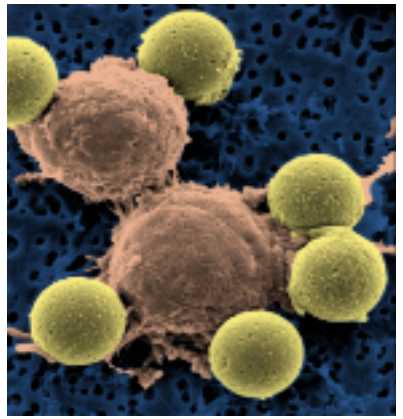
Autologous T Cells Transduced w/ Anti-CD19 Receptor Spliced to CD3 zeta and 4-1BB Signaling Domains



Lentiviral vector to deliver construct



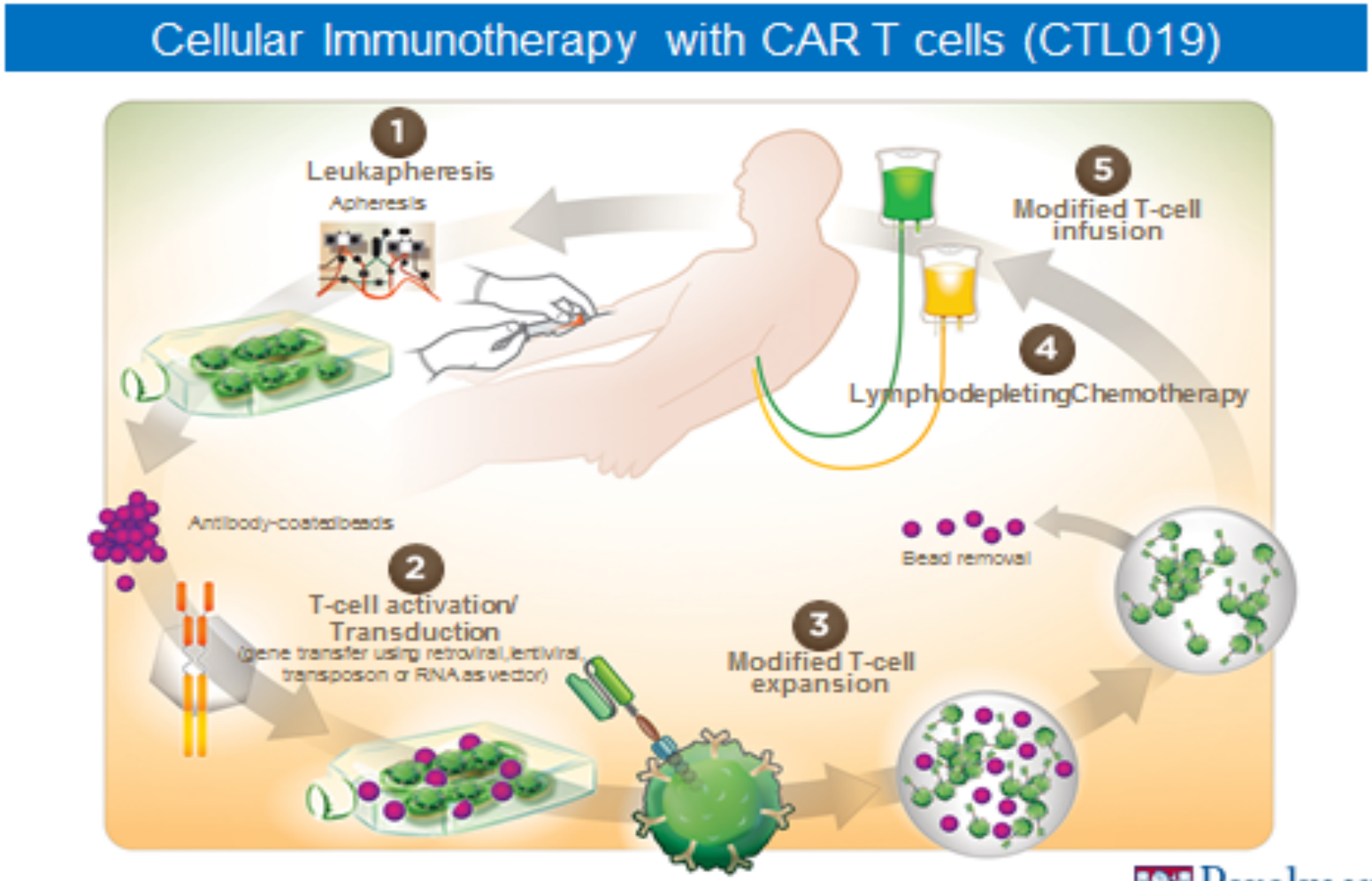
CD3-z and 4-1BB signaling domains augments proliferation and survival



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

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

Therapeutic Overview



Courtesy of Noelle Frye, MD

Perelman School of Medicine
University of Pennsylvania

Successes of CART19 Therapy

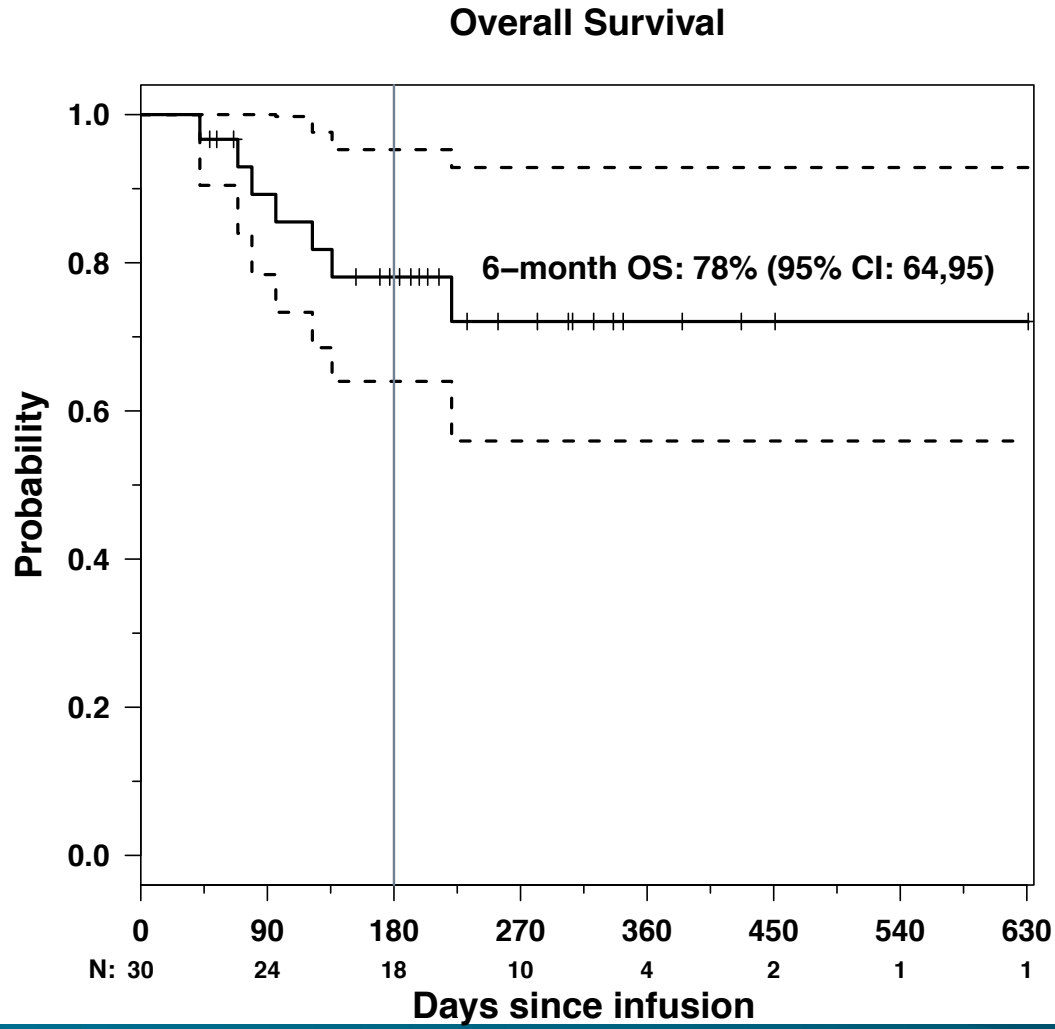
Ref	Program/ CAR	Population	Response
Acute Lymphoblastic Leukemia			
Maude et al. NEJM 2014	PENN 4-1BB	N=30(ALL) Peds&Adults	CR=90%
Davila et al. SciTrMed 2014	MSK CD28	N=16 (ALL) Adults	CR=88%
Lee et al. Lancet 2015	NCI CD28	N=21 (ALL) Peds&AYA	CR=67% Intent to Treat
Turtle et al. JCI 2016	Seattle 4-1BB	N=30 Adults	CR=93%
Non-Hodgkin 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%

ALL: Overall Response to CART19

Response	N=30	%
Complete Response	27/30	90%
No response	3/30	10%

Maude SL, Frey N. et al. N Engl J Med 2014;371:1507-1517.

CART19 for Rel/Ref ALL: Survival



Maude, Frey et al. NEJM 2014;371:1507-1517.

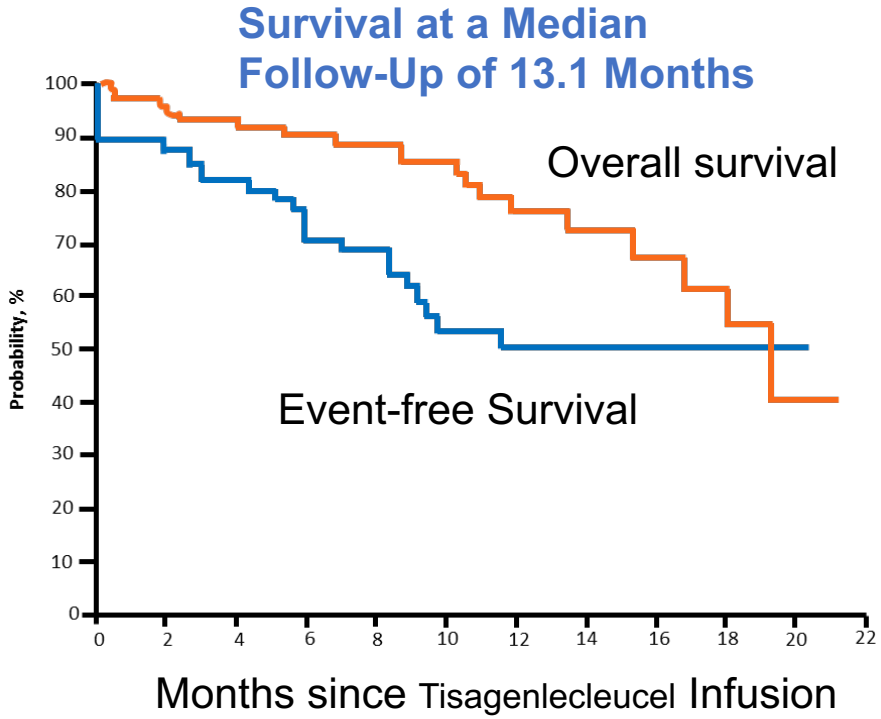
UNIVERSITY OF PENNSYLVANIA Abramson Cancer Center

2019 JADPRO Live THE ANNUAL APSHO MEETING

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 →

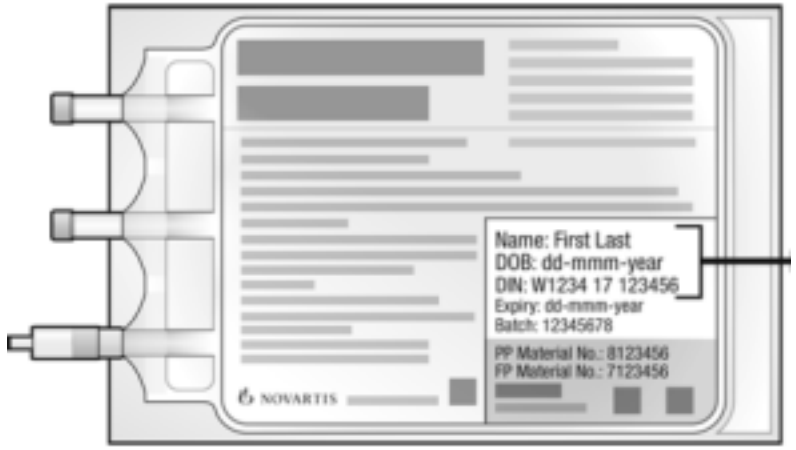


	Event Free Survival	Overall Survival
12 months	66%	76%
18 months	66%	70%
24 months	62%	66%

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.

First Gene Therapy Approval: Tisagenlecleucel

- FDA approved for B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse in the treatment of patients up to 25 years of age



- Approval date: August 30, 2017

- Lymphodepletion regimen:
  - Fludarabine 30 mg/m^2 D-6, D-5, D-4, D-3
  - Cyclophosphamide 500 mg/m^2 D-6, D-5

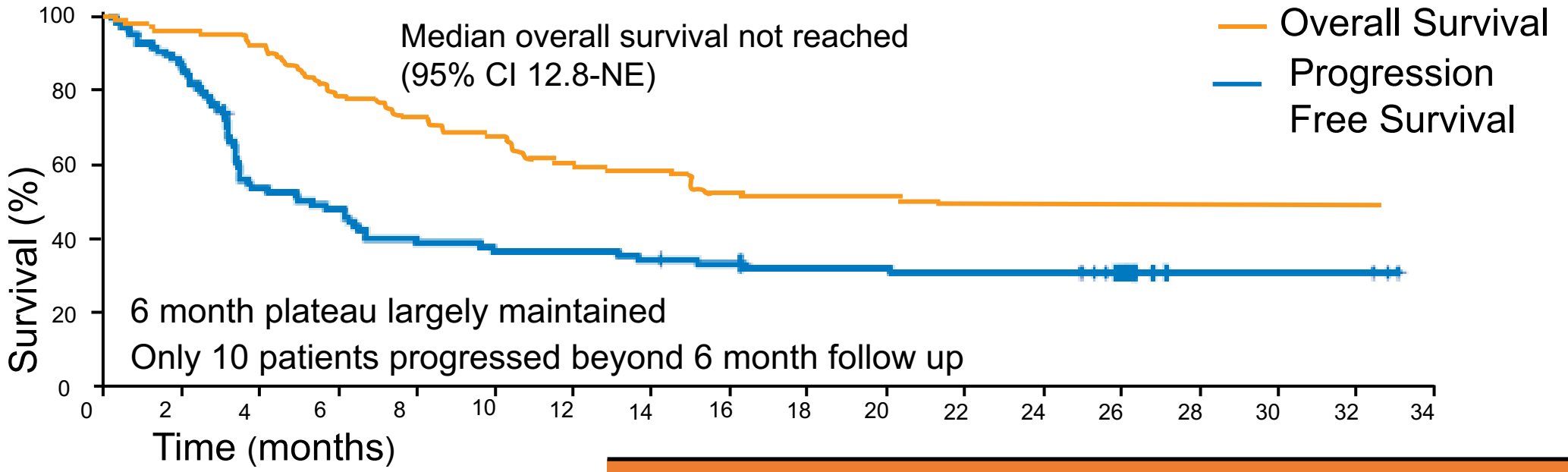
- Black box warning for CRS and neurotoxicity

Kymriah® [package insert]. East Hanover, New Jersey: Novartis Pharmaceuticals Corporation; 2017.

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%

ZUMA-1: Axicabtagene Ciloleucel in DLBCL Survival at a Median of 27.1 Months



Phase II trial of axicabtagene ciloleucel anti-CD19 CAR-T therapy in 101 patients with refractory large B-cell lymphoma

	Progression Free Survival	Overall Survival
6 months	49%	78%
12 months	44%	59%
24 months	39%	51%

Locke FL, et al. Lancet Oncol. 2019;20:31-42. Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.

Axicabtagene Ciloleucel

- FDA approved for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after 2 or more lines of systemic therapy - including DLBCL not otherwise specified, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma

- Lymphodepletion regimen:
  - Fludarabine 30 mg/m^2 D-5, D-4, D-3
  - Cyclophosphamide 500 mg/m^2 D-5, D-4, D-3

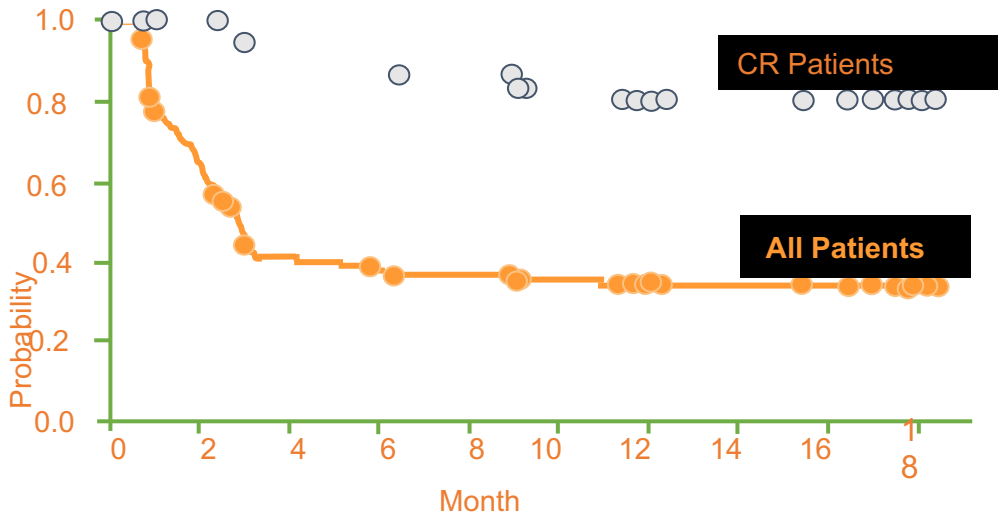
- Black box warning for CRS and neurotoxicity

Yescarta® [package insert]. Santa Monica, California: Kite Pharma, Inc; 2017

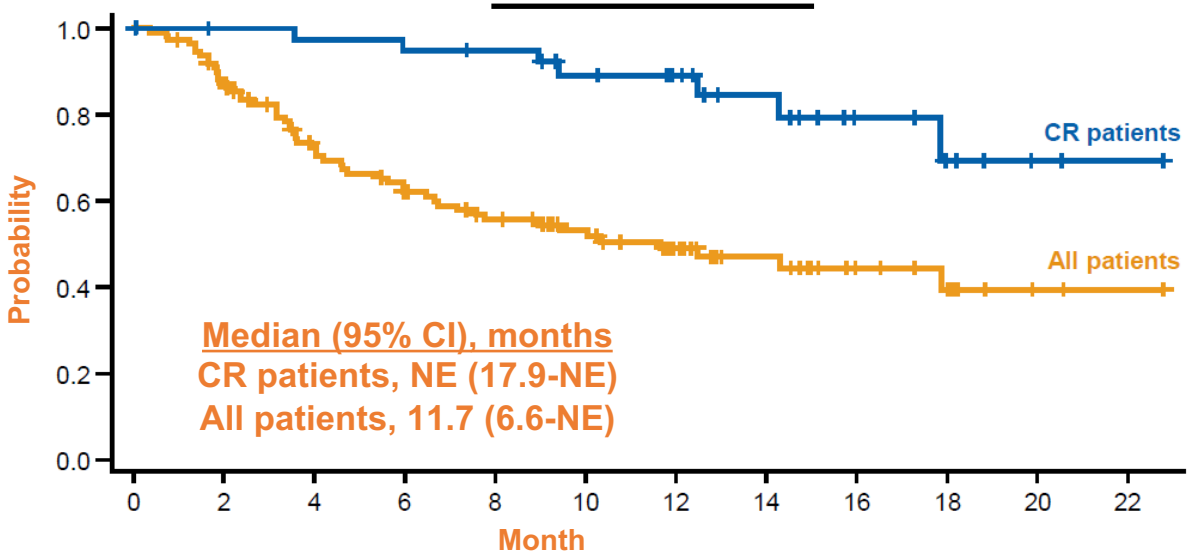
JULIET: Tisagenlecleucel in DLBCL

- 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.

Tisagenlecleucel: Second Indication

- FDA approved for adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy - including DLBCL not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma

- <u>Lymphodepletion regimen options</u>:
  - Fludarabine 25 mg/m^2 D-5, D-4, D-3
    Cyclophosphamide 250 mg/m^2 D-5, D-4, D-3
  - Bendamustine 90 mg/m2 D-4, D-3
    Previously experienced hemorrhagic cystitis with cyclophosphamide or demonstrate resistance to a cyclophosphamide regimen
  - Omit lymphodepletion if WBC $\leq$ 1x 10^9/L within one week of CAR T infusion

- Black box warning for CRS and neurotoxicity

Kymriah® [package insert]. East Hanover, New Jersey: Novartis Pharmaceuticals Corporation; 2017.

CD19 CAR T-Cell Products

	Axicabtagene Ciloleucel (axi-cel) *Kite Pharma (GILEAD)*	**Tisagenlecleucel (CTL019)** *Novartis*	**Lisocabtagene Maraleucel* (liso-cel)** *Juno Therapeutics*
US FDA Indication	Adult DLBCL	Ped/young adult ALL Adult DLBCL	Pending – adult DLBCL
CAR Type	CD19/CD28/CD3z	CD19/4-1BB/CD3z	CD19/EGFRt/4-1BB/CD3z
Costimulatory Domain	CD28	4-1BB (CD 137)	4-1BB (CD 137)
scFv	FMC63	FMC63	FMC63
Vector	Retrovirus	Lentivirus	Lentivirus
Defined Cells	No	No	CD4:CD8
Pivotal Trial	ZUMA-1 (LBCL)	ELIANA (ALL), JULIET (DLBCL)	TRANSCEND (LBCL)

***Not FDA-approved**

Locke FL, et al. Lancet Oncol. 2019;20:31-42. Neelapu SS, et al. N Engl J Med. 2017;377:2531-2544.
Schuster SJ, et al. N Engl J Med. 2019;380:45-56. Abramson JS, et al. J Clin Oncol 36, 2018 (suppl; abstr 7505).

Summary: CART19 in CD19+ Disease

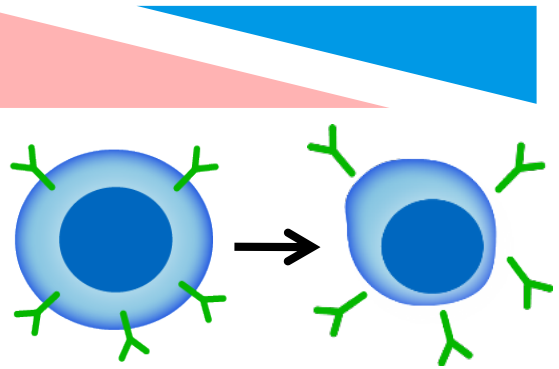
- 80-90% CR rate in rel/ref ALL & 50% ORR in CLL
  - MRD negative
  - Successful bridge to ALLO SCT
  - Some pts with prolonged remissions from CART19 alone
- CAR T cells can persist for >48 months (Penn experience)
  - Cells remain functional
  - Correlates with remission & B cell aplasia (IVIG replacement)
- CRS is most significant toxicity
  - Responsive to supportive care and anti-cytokine therapy
- Relapses
  - CD19 negative: combination strategies/baseline predictors?
  - CD19 positive: loss of persistence

1. Porter D, et al. ASH 2013; Abstracts 873 and 4162.

Designing a Myeloma CAR: Candidate antigen targets

CD19 expression (B cell antigen)

BCMA expression (plasma cell antigen)



Memory B cell

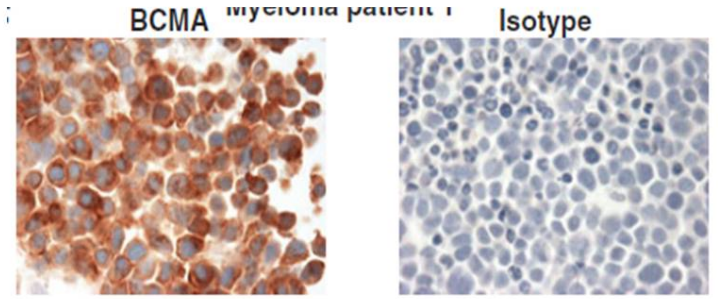
Plasma cell

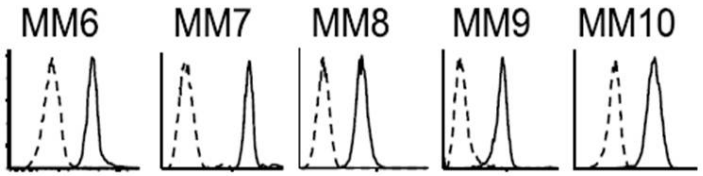
Rare putative myeloma stem cell population

Dominant clinical myeloma cell population

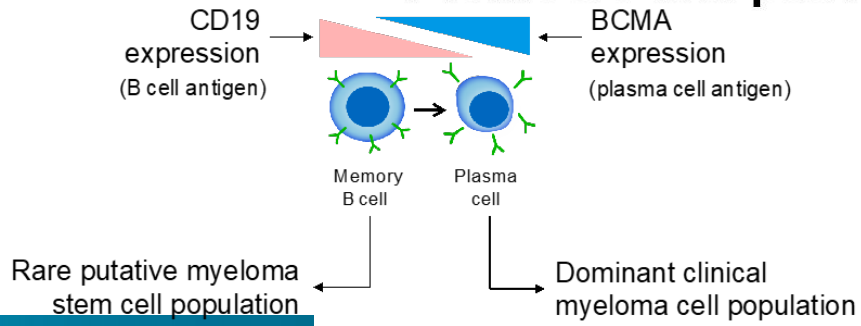
BCMA (*B-cell Maturation Antigen*)

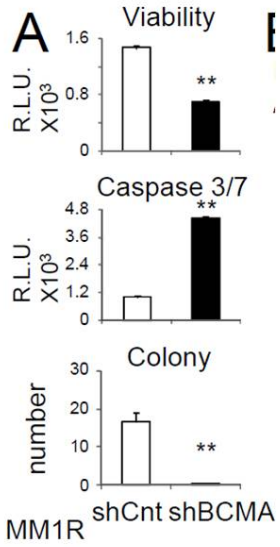
- **Receptor for BAFF (Blys) and APRIL**
- **Expressed on plasma cells, some mature B cell subsets, and plasmacytoid DC's**
  - Maintains plasma cell homeostasis
- **Highly expressed on myeloma cells**
- **Soluble BCMA in patient serum**

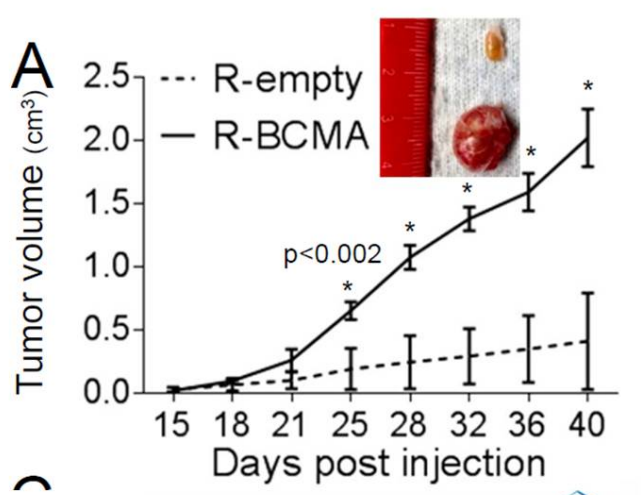




- **Promotes MM pathogenesis**







Frigyesi et al, Blood 2014; Tai et al, Blood 2014; Carpenter et al, Clin Can Res 2013; Tai et al, Blood 2016

BCMA CAR T cells – initial studies, refractory pts

Trial	n	Condi-tioning	# lines	% hi risk†	ORR	ORR (optimal doses)	VGPR/CR (optimal doses)
NCI[1]	26*	Cy/Flu	7.5	42%	58%	81% (13/16)	63% (10/16)
Penn[2]	25	None or Cy	7	76%	48%	64% (7/11)	36% (4/11)
Bluebird[3]	43	Cy/Flu	7.5	40%	77% (30/39)	96% (21/22)	86% (19/22)
Janssen[4]	57	Cy	NA	NA	88%		78%

*2 treated twice; counted separately for response. † FISH +t(4;14), t(14;16), del 17p *excluded high tumor burden in last 14 pts. NR = not reported

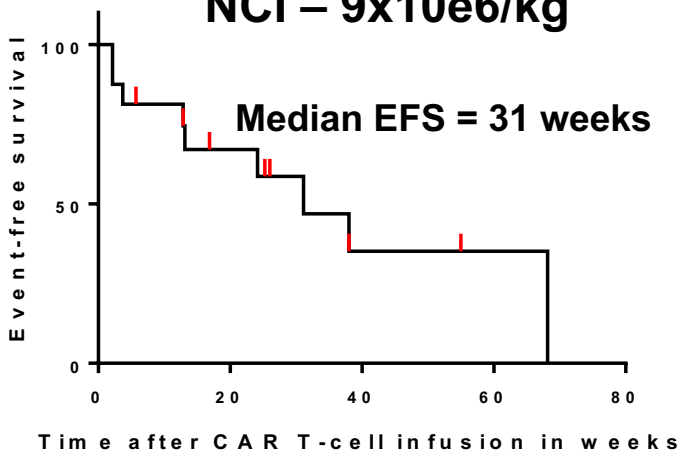
Trial	n	CRS %	CRS G3-4 %	Neurotox %	Neurotox G3-4 %	Toci
NCI[1]	26*	73%	23%	NR	12%	19%
Penn[2]	25	88%	32%	32%	12%	28%
Bluebird[3]	43	63%	5%	33%	2%	21%
Janssen	57	76%	7%	42%	2%	

[1]Ali, Blood 2016 and Brudno, J Clin Oncol 2018;
[2]Cohen, JCI 2019
[3]Raje, NEJM 2019 ;
[4]Zhao. ASH 2018

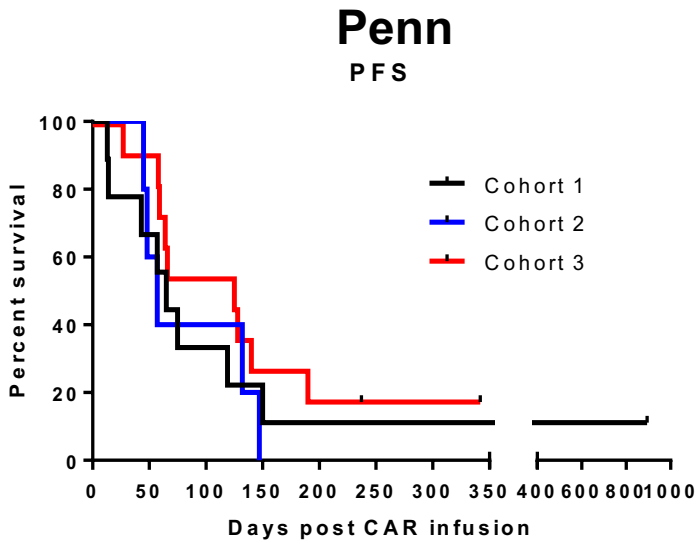
BCMA CAR T cells – lessons from initial studies

- Probably not curative in refractory patients

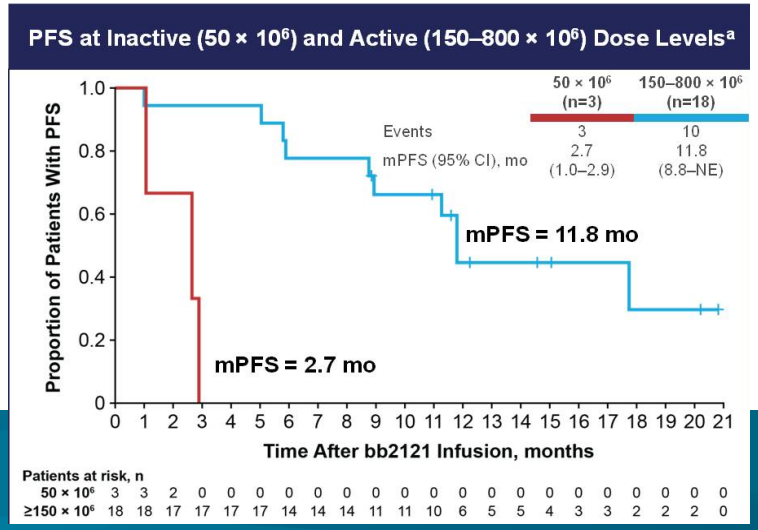
NCI – 9x10e6/kg



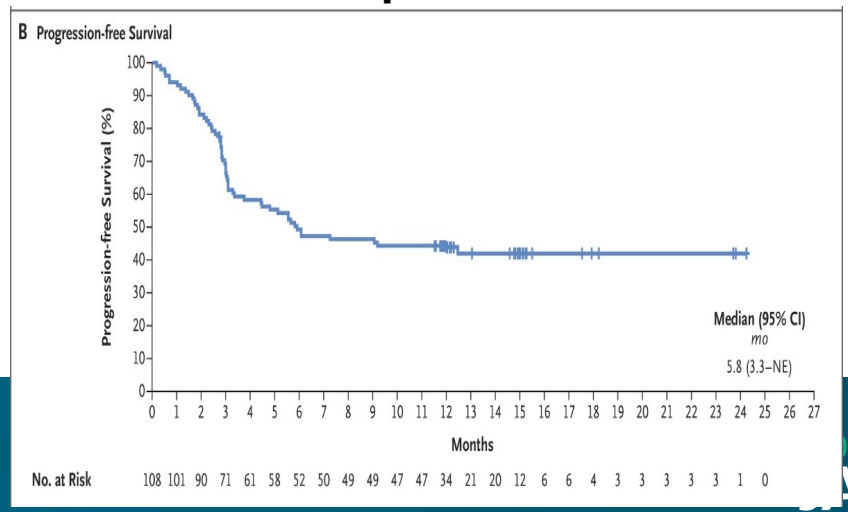
Penn



Bluebird – dose escalation



DLBCL ph2 Yescarta



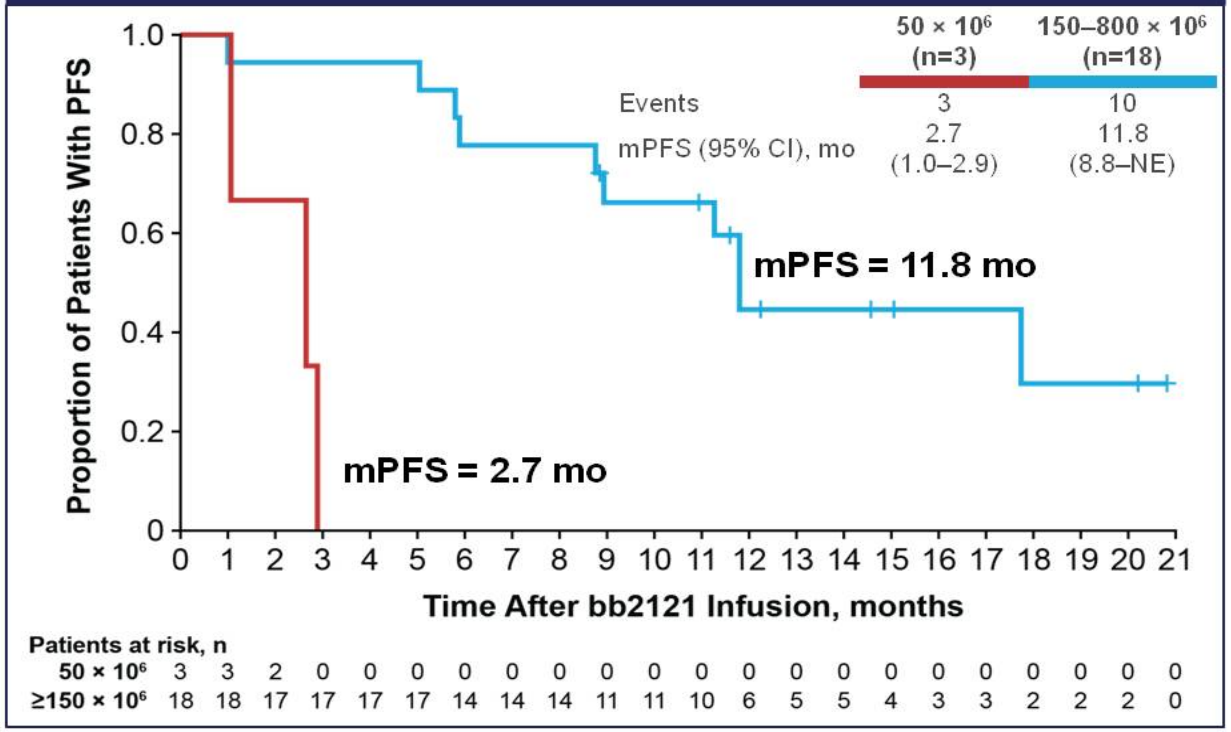
[1]Ali, Blood 2016 and Brudno, J Clin Oncol 2018; [2]Cohen, JCI 2019 [3]Raje, NEJM 2019 ;[4]Zhao. ASH 2018

PROGRESSION-FREE SURVIVAL

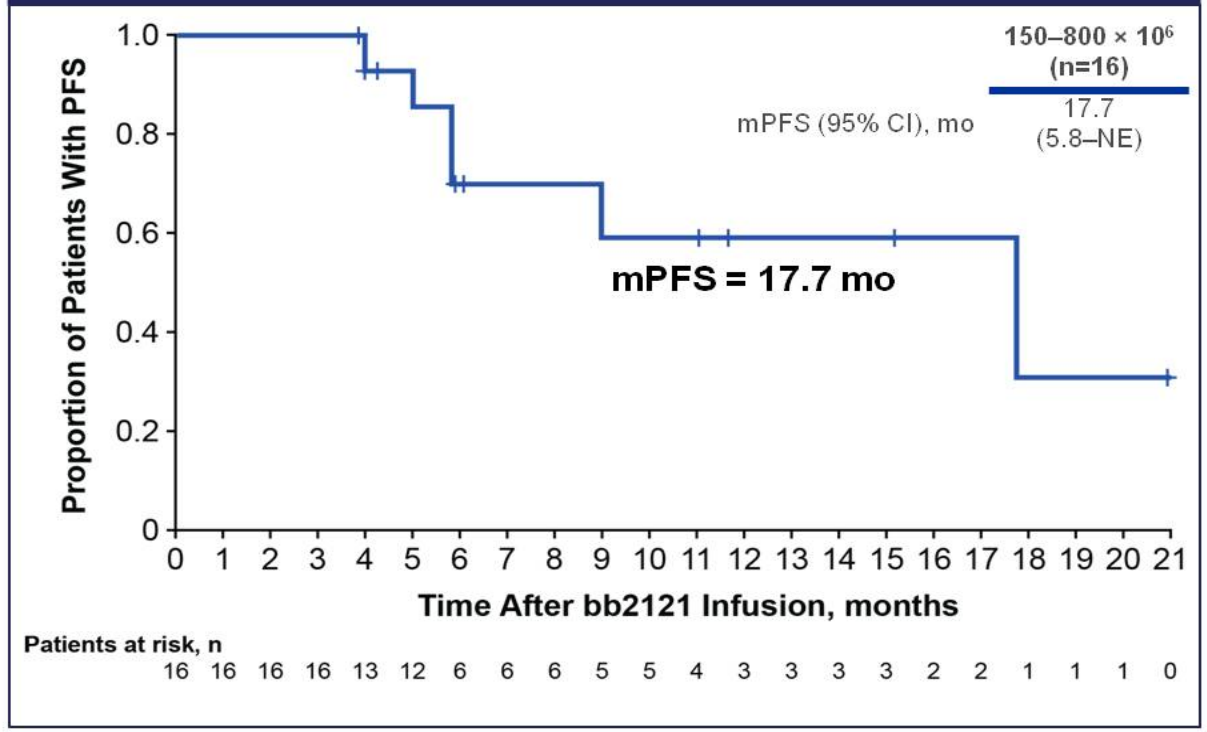
Interpretation:
Dose matters, Not Fixing everyone

- mPFS of 11.8 months at active doses (≥150 × 10^6 CAR+ T cells) in 18 subjects in dose escalation phase
- mPFS of 17.7 months in 16 responding subjects who are MRD-negative

PFS at Inactive (50 × 10^6) and Active (150–800 × 10^6) Dose Levels[a]



PFS in MRD-Negative Patients



Data cutoff: March 29, 2018. Median and 95% CI from Kaplan-Meier estimate. NE, not estimable. [a]PFS in dose escalation cohort.

Raje, NEJM 2019

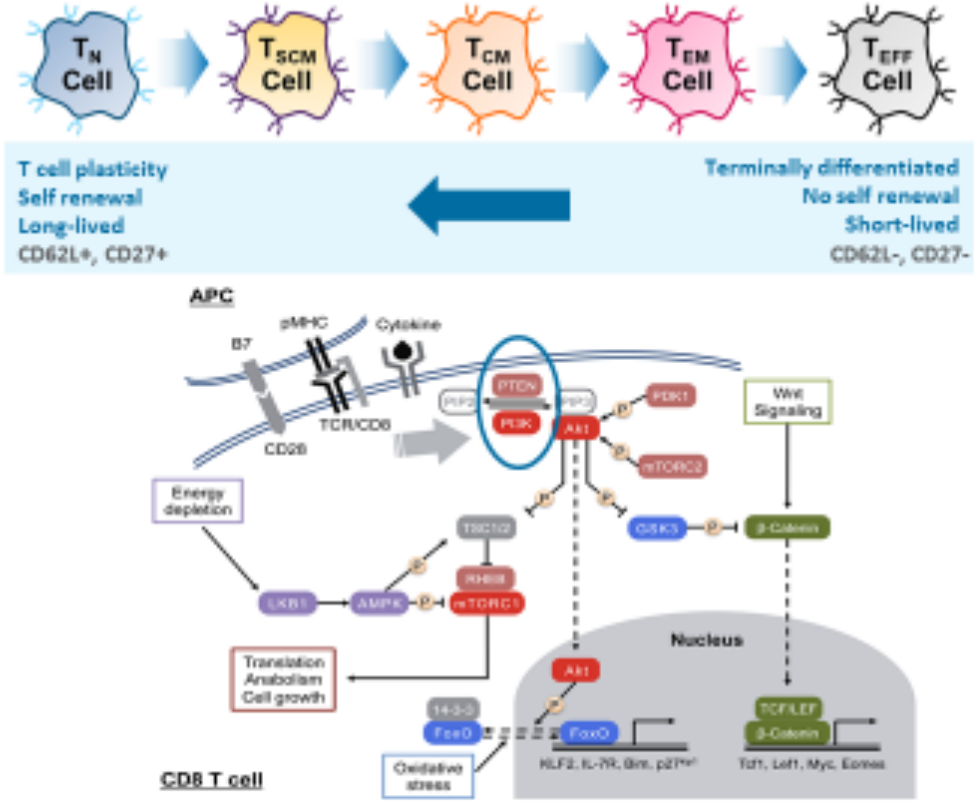
Designing Better BCMA CARS

- **Targets**
  - Single vs multiple
- **Constructs**
  - antigen recognition
  - stimulatory molecules
- **Vectors**
  - Viral
  - Non-viral approaches
- **Dose**
- **Off switches**
- **Lympho-depletion**
- **Single vs serial infusions**
- **Patient selection**
  - Test for target
  - Early vs heavily pre-treated disease
  - Early vs dysfunctional T-cells
  - Early vs late dysfunctional host

CART-BCMA manufacturing with PI3 kinase inhibition

bb21217: Next-Generation Anti-BCMA CAR T Cell Therapy Product for Multiple Myeloma

- bb21217 uses the same CAR construct design as bb2121[1]
- bb21217 is cultured with PI3 kinase inhibitor, bb007, to enrich for T cells displaying a memory-like phenotype
- CAR T cells enriched for this phenotype may persist and function longer than non-enriched CAR T cells[2]
- Persistence of functional CAR T cells after infusion may be one determinant of duration of response



BCMA, B-cell maturation antigen; PI3K, phosphoinositide 3 kinase.

1. Friedman et al. *Hum Gene Ther* 2018;29:585-601. 2. Fraietta IA, et al. *Nat Med*. 2018 May;24:563-571

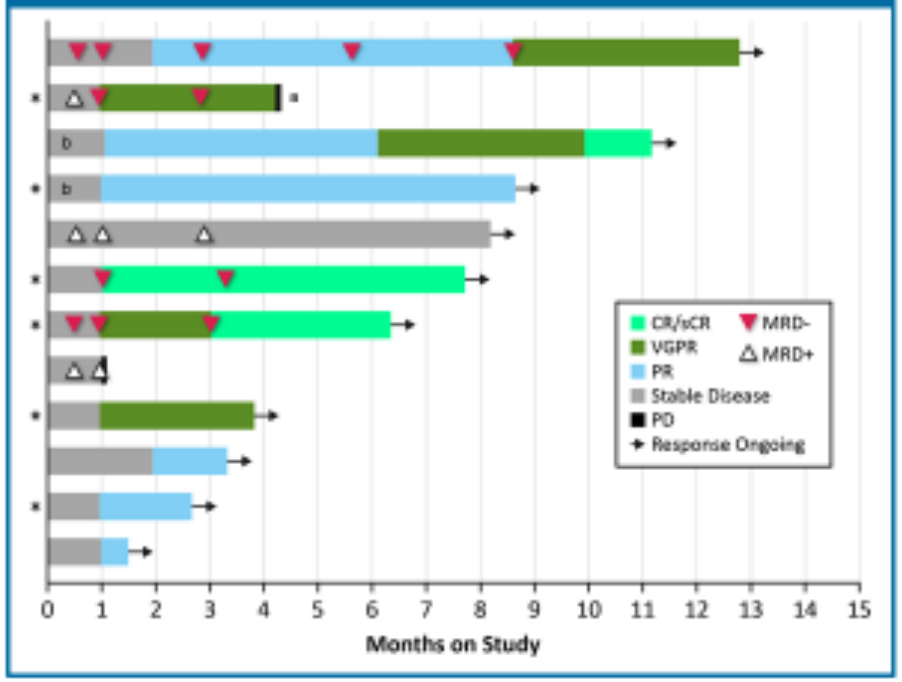
Shah et al, ASH 2018, #488

CART-BCMA manufacturing with PI3 kinase inhibition

- Toxicities similar to bb2121 (CRS, neurotox)
- ?any difference in memory phenotype, persistence?

Clinical Responses and Duration of Response at the 150 × 10^6 CAR+ T Cell Dose

Clinical Responses Over Time



CR, complete response; MRD, minimal residual disease; ORR, objective response rate; PR, partial response; VGPR, very good partial response. [a]Patients with high tumor burden. [b]Progression based exclusively on appearance of new bone lesions. [c]MRD status not available. [d]Includes unconfirmed responses. [e]Patients with ≥PR and valid MRD assessments. [f]Two MRD-neg responses at 10^{-4} and 2 at 10^{-5} sensitivity level by Adaptive next-generation sequencing. [g]Among 10 responders with ≥PR.

Clinical Response

	bb21217-Treated (N=12)
ORR,[c] n (%) [95% CI]	10 (83.3) [51.6, 97.9]
sCR/CR	3 (25)
≥VGPR	6 (50)
MRD status in bone marrow, n	
MRD-evaluable responders[d]	4
MRD-neg	4[e]
Median time to first response (min, max),[c,f] mo	1 (1, 2)
Median time to best response (min, max),[c,f] mo	1 (1, 10)
Median follow-up duration (min, max), mo	5.9 (1.0, 11.8)

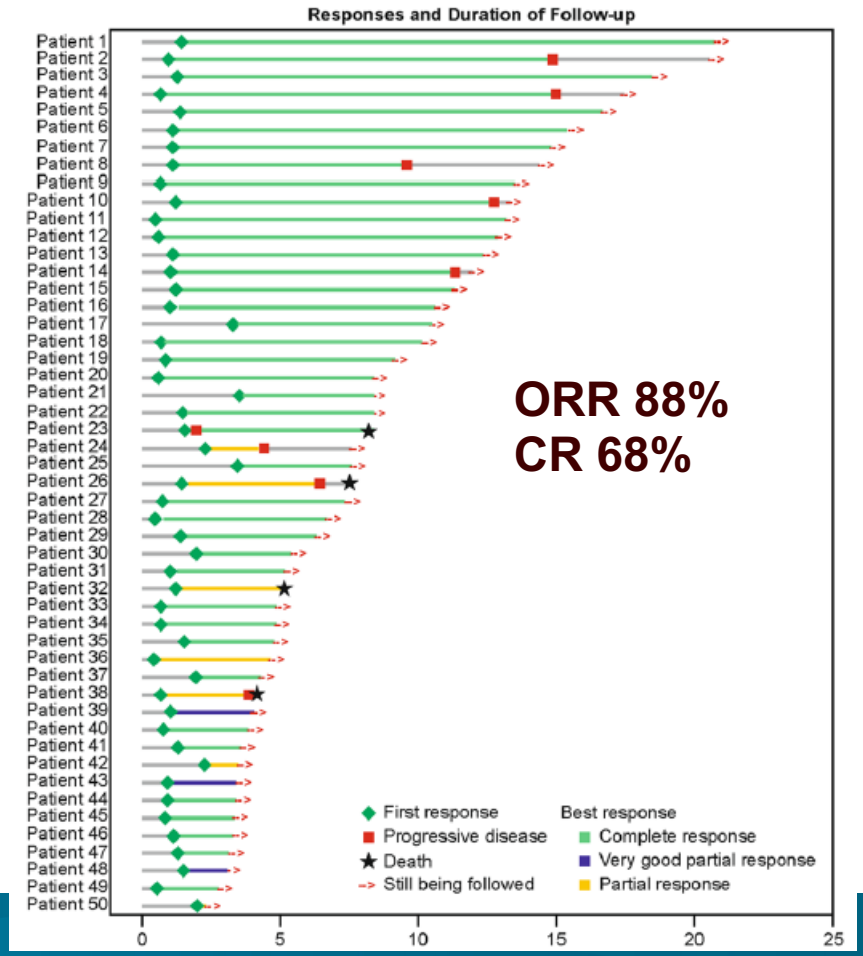
- 10/12 patients (83%) achieved an objective response at the first tested dose (150 × 10^6 CAR+ T cells)
- Responses deepening over time; CR achieved as late as month 10
- Responses ongoing in all but 1 responder; first patient dosed continues in response >1 year after treatment
- 100% MRD negativity in 4/4 responders evaluable for MRD status; 2/2 non-responders were MRD positive

Data Extract: 18OCT2018

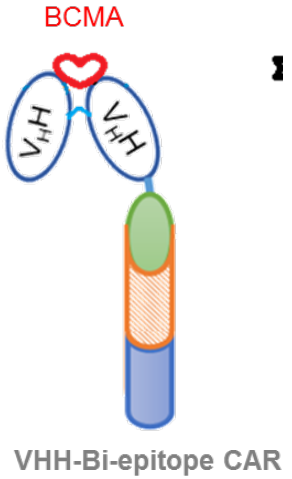
Shah et al, ASH 2018, #488

Legend Biotech: Phase 1 LCAR-B38M (BCMA CAR T cells)

- Single institution experience (n=57)
- CD3/41BB dual-binding CAR, Cy conditioning, med 3 prior

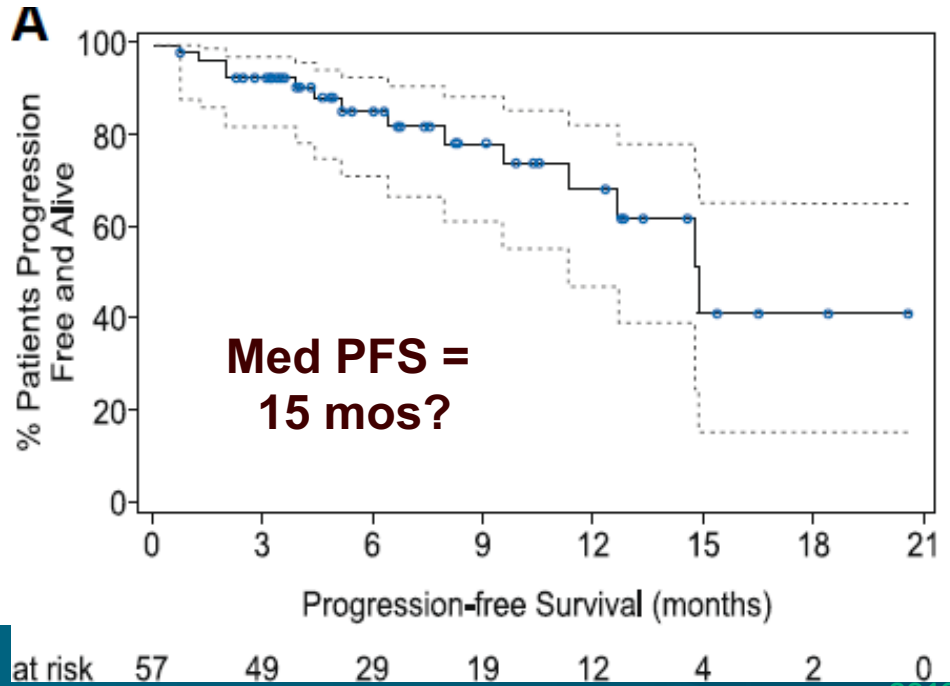


ORR 88%
CR 68%



VHH-Bi-epitope CAR

CRS 90% (7% Gr 3-4)
Neurotox 2% (Gr 1)



Med PFS = 15 mos?

Zhao et al, ASH 2018, #955 and J Hematol Oncol 2018

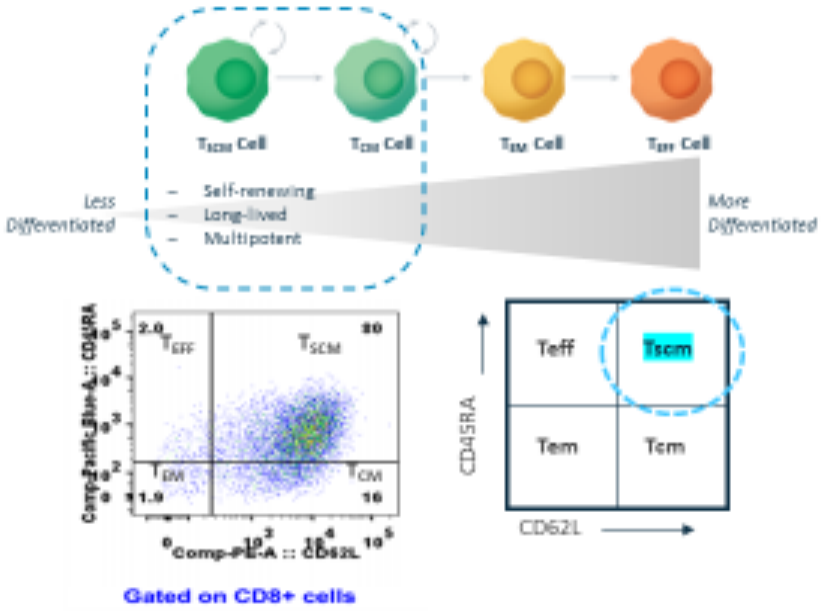
Transposon-based BCMA CAR construct

- Non-viral gene delivery system, larger cargo capacity
  - Cheaper/faster manufacturing, positive selection gene, suicide gene

P-BCMA-101: Comprised of a High Percentage of Desirable T_{SCM} Cells

We believe T_{SCM} cells in product is the key to increase duration of response and reduce toxicity

- **high percentage of T_{SCM}** cells is a distinct advantage
- **piggyBac™ preferentially transposes in T_{SCM} cells**
- **T_{SCM} cells engraft and live longer** than more differentiated T cells
- **T_{SCM} cells** can produce potentially unlimited waves of effector cells
- T_{SCM} cells should lead to **better duration of response**, potential for **re-response and efficacy in solid tumors,** with more gradual tumor killing producing **less toxicity**

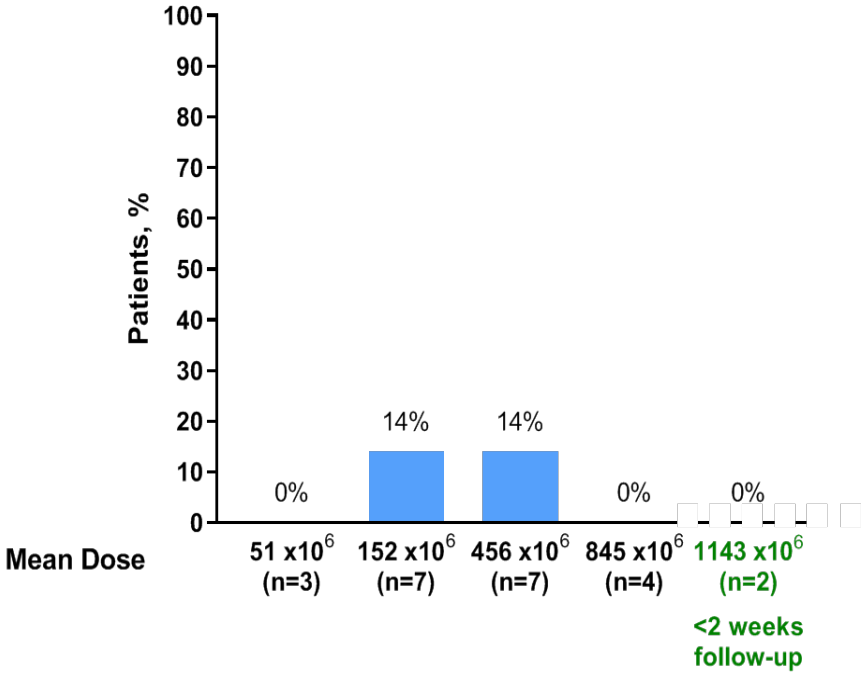


Gregory et al, ASH 2018, #1012

Transposon-based BCMA CAR construct

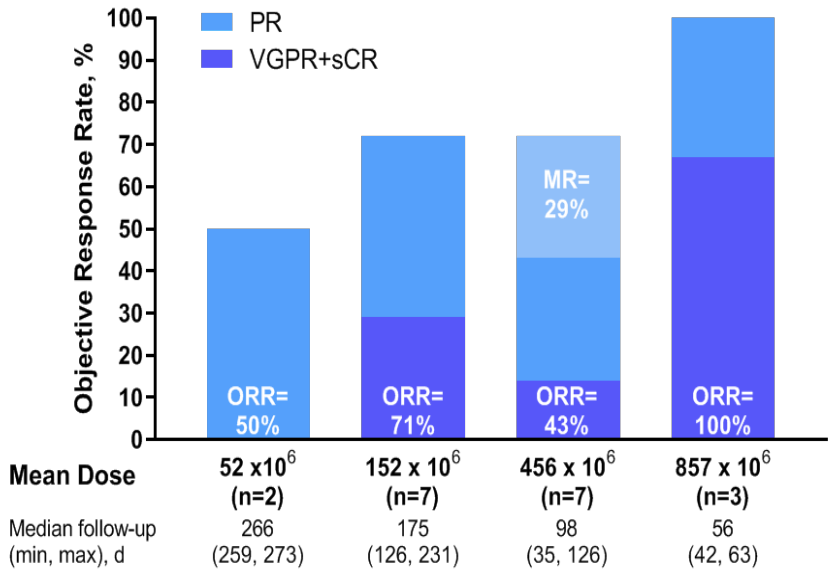
Slower in vivo expansion (peak day 14-21)

Cytokine Release Syndrome By Dose Level



1 neurotoxicity

Tumor Response in Evaluable Patients by Dose

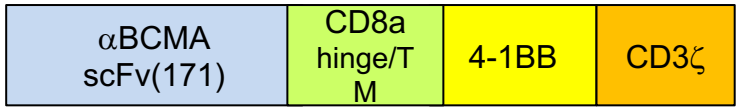


ORR = 63% (12/19 evaluable)

Gregory et al, ASH 2018, #1012

MSKCC/Juno Vectors in clinical trials

- MCARH171
  - Retrovirus
  - No Pre-defined CD4:CD8 ratio



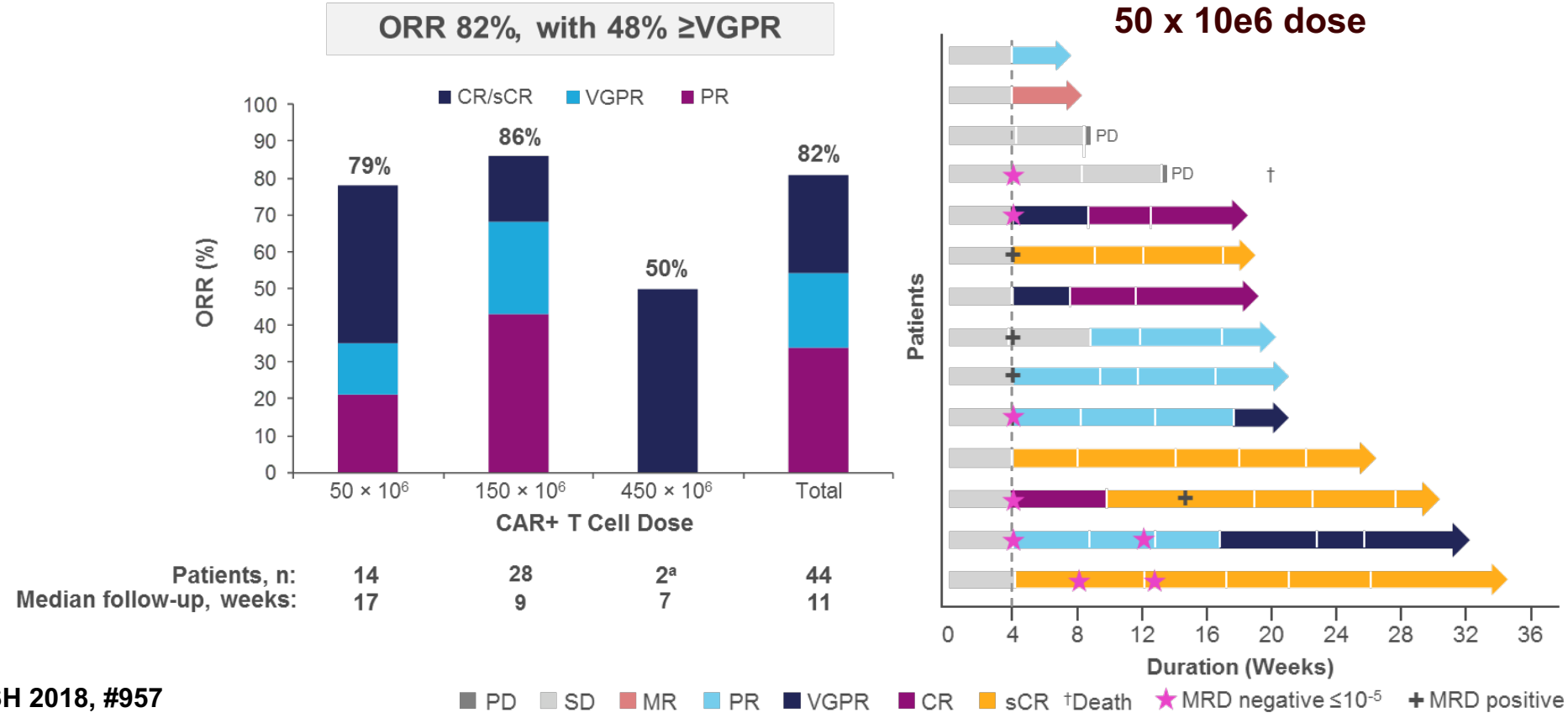
- JCARH125 (EVOLVE)
  - Lentivirus
  - 1:1 CD4:CD8 ratio prior to transduction and expansion

- FCARH143
  - Lentivirus
  - 1:1 CD4:CD8 ratio after trans

ASH 2018 abstracts 959, 957 and 1011.

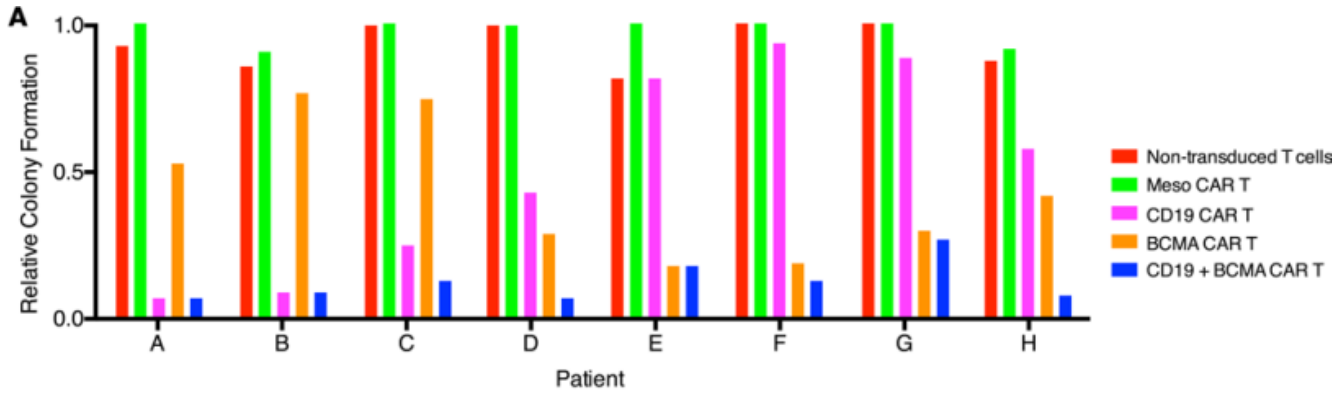
Ph 1/2 JCARH125 (defined CD4:CD8 pre-manufacturing)

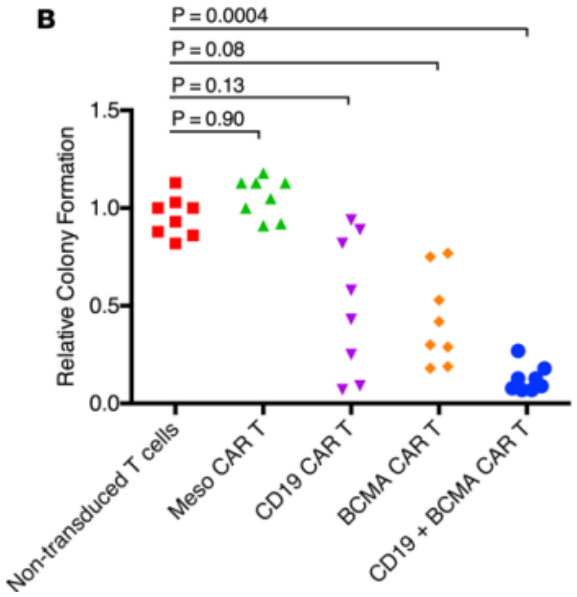
- CRS 80% (Gr 3-4 9%)
- Neurotox 25% (Gr 3-4 7%)



Mailankody et al, ASH 2018, #957

Dual BCMA/CD19 Directed CAR Myeloma Trial





- Correlates of favorable clinical outcome
  - peak CTL019 frequency in bone marrow
  - emergence of humoral and cellular immune responses against the stem-cell antigen Sox2.
- Ex-vivo treatment of primary myeloma samples with a combination of CTL019 and BCMA CAR T
  - reliably inhibited myeloma colony formation in vitro while either alone inhibited colony formation inconsistently.

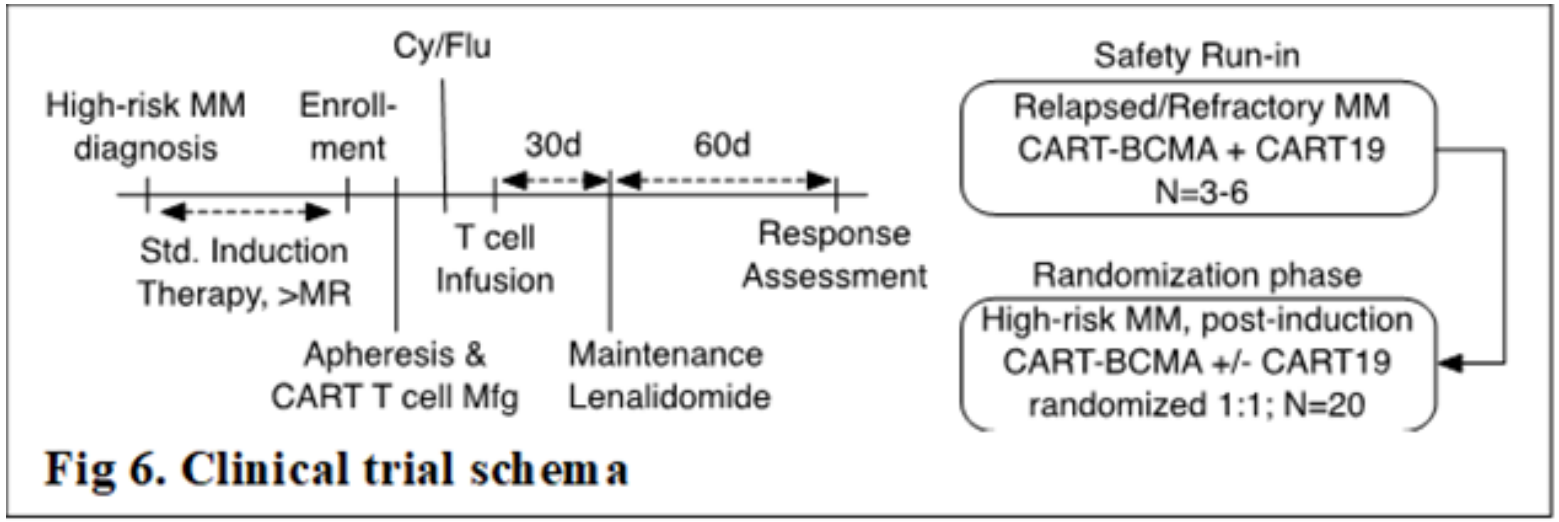


Fig 6. Clinical trial schema

Garfall et al, NEJM 2015, JCI Insight 2018

A combination of humanized anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial (21 pts)

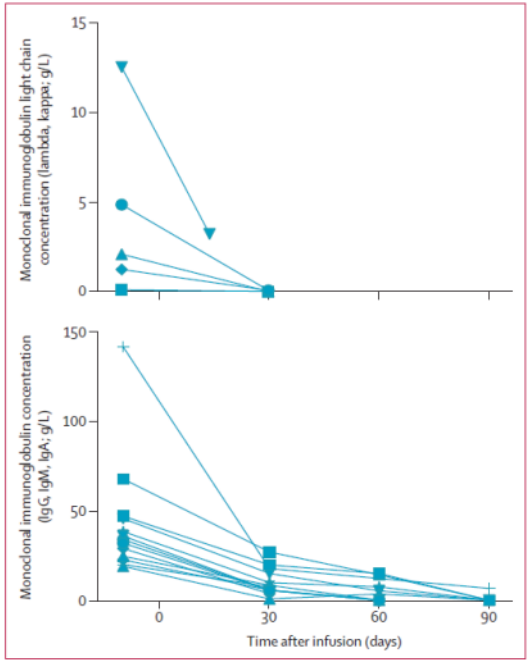
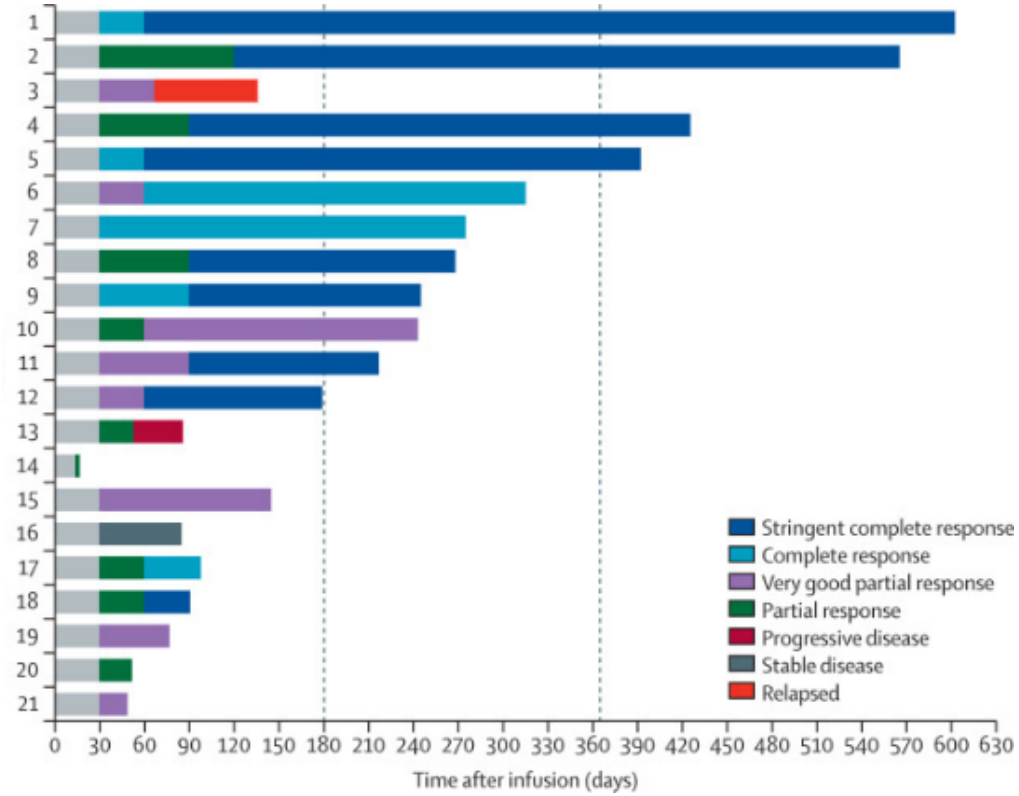


Figure 3: **Changes in monoclonal immunoglobulin concentration from baseline**
Each line represents a single patient.



	Cytokine release syndrome (grade)	Neuro-toxicity	Response at 1 month	Minimal residual disease-negativity		Final outcome (days of follow-up)
				Yes or No	First confirmed time after infusion, months	
1	Yes (1)	No	Complete	Yes	2	Stringent complete response (602)
2	Yes (1)	No	Partial	Yes	1	Stringent complete response (565)
3	Yes (2)	Yes	Very good partial	Yes	1	Relapsed on day 67 died on day 136
4	Yes (1)	No	Partial	Yes	1	Stringent complete response (425)
5	Yes (3)	Yes	Complete	Yes	1	Stringent complete response (392)
6	Yes (1)	No	Very good partial	Yes	1	Complete response (315)
7	Yes (1)	No	Complete	Yes	1	Complete response (275)
8	Yes (1)	No	Partial	Yes	1	Stringent complete response (268)
9	Yes (2)	No	Complete	Yes	1	Stringent complete response (245)
10	Yes (1)	No	Partial	Yes	1	Very good partial response (243)*
11	Yes (2)	No	Very good partial	Yes	1	Stringent complete response (217)
12	Yes (1)	No	Very good partial	Yes	1	Stringent complete response (179)
13	No	No	Partial	No	..	Progressed on day 53 and received salvage therapy (86)†
14	Yes (2)	No	Death	No	..	Partial response on day 14, died of cerebral haemorrhage (17)
15	Yes (1)	No	Very good partial	Yes	1	Very good partial response (145)*
16	Yes (1)	No	Stable disease	No	..	Stable disease (85)
17	No	No	Partial	No	..	Complete response (98)
18	Yes (1)	No	Partial	Yes	1	Stringent complete response (91)
19	Yes (2)	No	Very good partial	Yes	1	Very good partial response (77)*
20	Yes (2)	No	Partial	Yes	1	Partial response (52)*
21	Yes (2)	No	Very good partial	Yes	1	Very good partial response (49)*

*The immunofixation electrophoresis is still weakly positive. †Patient lost to follow-up after 86 days.

Table 2: **Clinical responses and serious toxicities by patient**

Designing a Myeloma CAR: Candidate antigen targets

- The classics
  - CD138
  - CD38
  - CD56
  - Kappa light chain
  - CD19

- **The new models:**
  - Lewis Y
  - CD44v6
  - MAGE A3
  - NY-ESO-1
  - CS1/SLAMF7
  - **BCMA**
  - Integrin beta 7
  - FcRH5
  - CD48
  - CD46
  - CD229
  - GPRC5D

CAR T cells for MM in 2018

Antigen	Trial Site/Company	Accrual
BCMA	National Cancer Institute	completed (n=26)
BCMA	University of Pennsylvania / Novartis	completed (n=25)
BCMA	Multi-site phase 1/ Bluebird	ongoing (n=21 reported)
BCMA	Multi-site phase 2/ Bluebird	ongoing
BCMA	Multi-site phase 1 / Bluebird (bb21217 product)	ongoing
BCMA	Multi-site phase 1/2, Nanjing Legend	ongoing (n=19 reported)
BCMA	Memorial Sloan-Kettering / Juno	ongoing (n=6 reported)
BCMA	Fred Hutchinson / Juno	ongoing
BCMA	Multi-site phase 1/2, Juno	ongoing
BCMA	Multi-site phase 1, Poseida	ongoing
BCMA	Multi-site phase 1, Kite	ongoing
BCMA	Multiple hospital sites in China	ongoing
BCMA	Multi-site phase 1/2, Autolus Limited	ongoing
BCMA	Virginia Cancer Specialists, Cartesian Therapeutics	ongoing

Antigen	Trial Site/Company	Accrual
CD19	University of Pennsylvania / Novartis	completed (n=10)
CD19 + BCMA	University of Pennsylvania / Novartis	open 2018
CD19 + BCMA	Soochow University, China	ongoing (n=10 reported)
CD138	General Hospital of PLA, China	completed (n=5)
CD138	Soochow University, China	ongoing
Kappa LC	Baylor University	completed (n=7 MM)
CD38	Multi-site phase 1, Sorrento Therapeutics	ongoing
CD38	Shenzhen Geno-Immune Medical Institute, China	ongoing
CD38	n/a	pre-clinical
SLAMF7/ CS1	n/a	pre-clinical

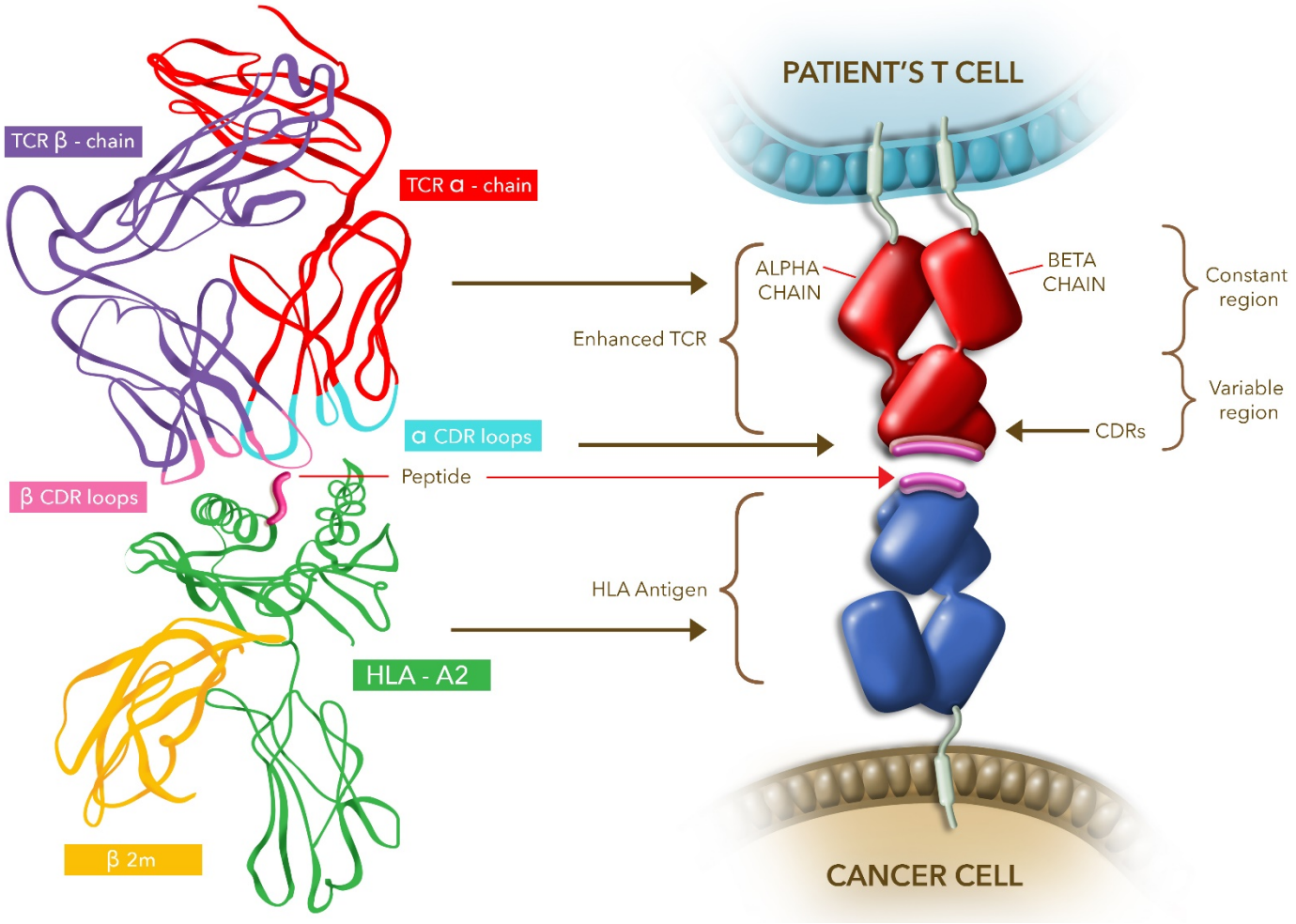
www.clinicaltrials.gov, March 2018

Cancer Testis Antigens (NY-ESO-1, LAGE-1)

- Expressed in a wide variety of cancers, including multiple myeloma
- Good immunotherapy targets due to limited expression on normal somatic tissue
- Restricted expression decreases the likelihood of ‘on-target off-tumor’ effects
- The frequency of CTA expression tends to increase with cancer stage and recurrence
- NY-ESO-1 and LAGE-1a have been detected at higher levels in advanced multiple myeloma

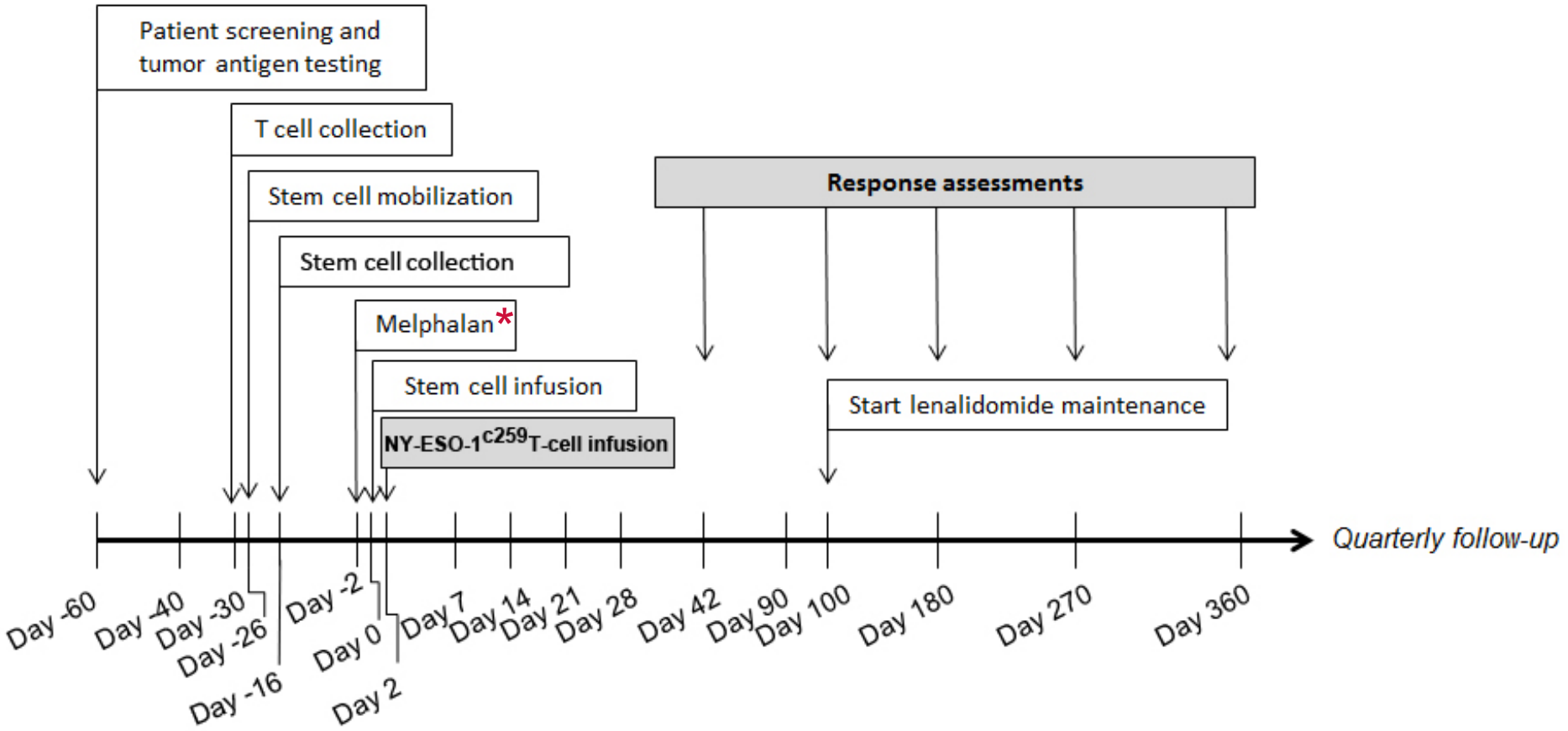
Ghafouri-Fard et al, *Iran J Cancer Prev* 2015
van Rhee et al, *Blood* 2005
Gjerstorff et al, *Oncotarget* 2015

NY-ESO-1^{c259}TCR : Enhanced Affinity (PENN, MARYLAND, ADAPTIMMUNE/GSK)



- Lentiviral vector. All domains of the natural TCR are intact, with no added intracellular signaling domains
- The engineered TCR targets NY-ESO-1 and LAGE-1a, as the same epitope (*SLLMWITQC*) is present on both CTAs
- The CDRs (complementary determining regions) are modified to enhance the recognition of the *SLLMWITQC* peptide in the context of HLA-A*02

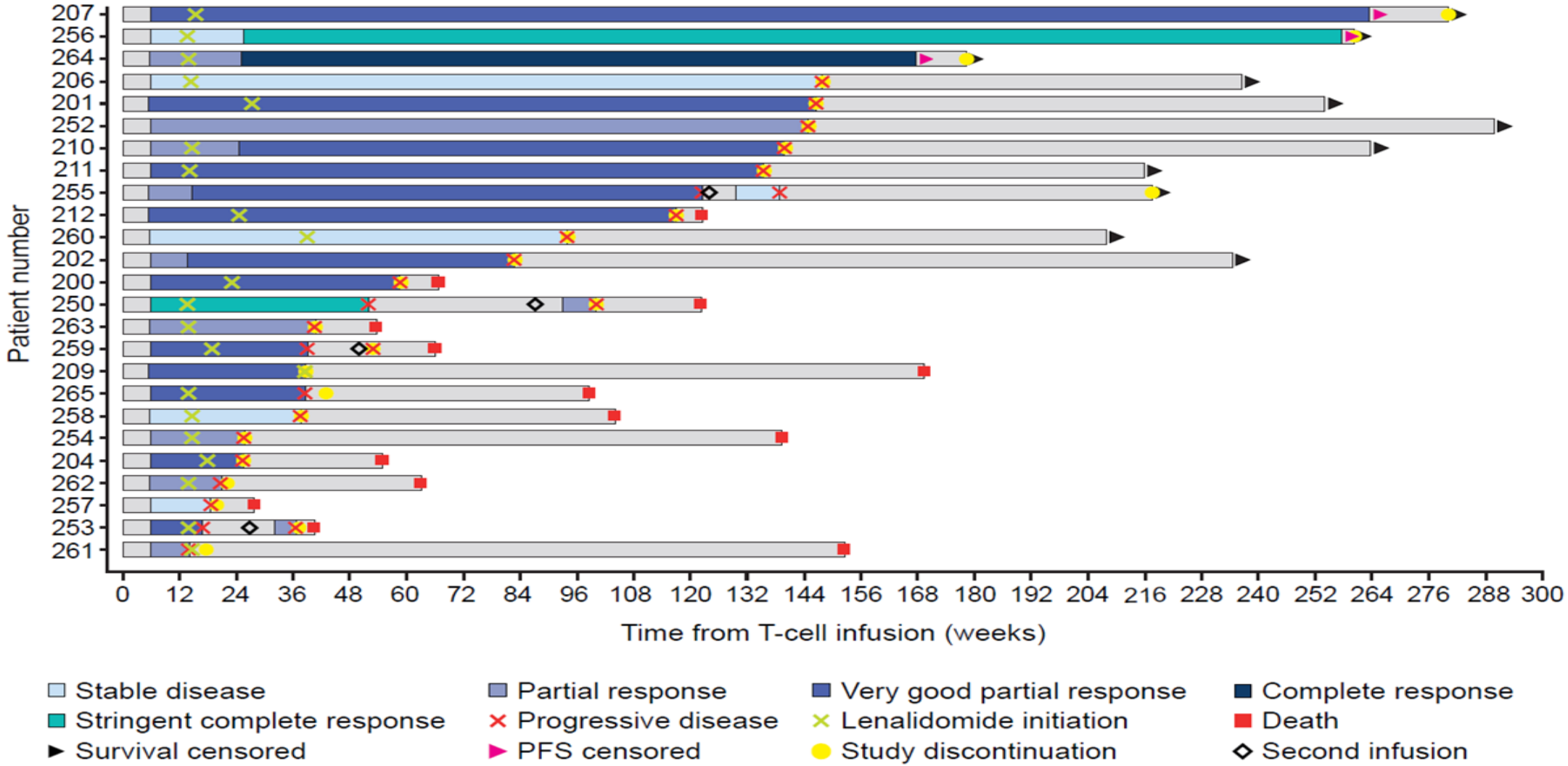
Overview of Study Design



* High dose: 200mg/m^2

Rapoport et al, *Nat Med* 2015

Response Summary



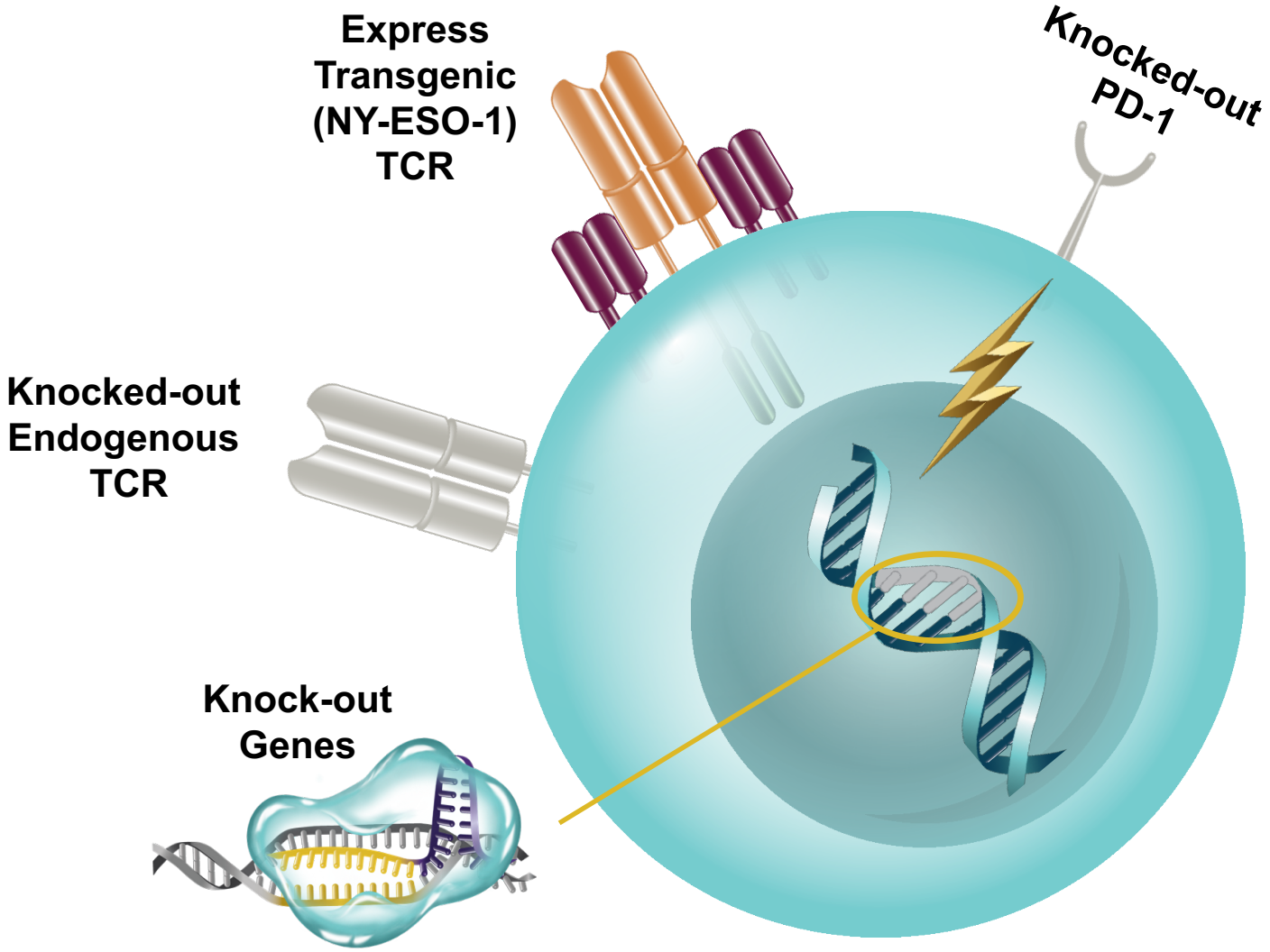
Conclusions

- NY-ESO-1^{c259}T-cell therapy in the setting of ASCT has promising efficacy and acceptable safety
- Long-term survival demonstrated in a refractory population
- It is possible to achieve negative MRD with this therapy
- TCR-transduced T-cells persist long term and are not exhausted
- Persisting cells produce multiple cytokines in response to antigen
- Persisting cells include highly differentiated effector subsets and a population of self-renewing stem cell/memory cells
- BUT inconclusive:
- Partnered with MEL 200 ASCT; no long-term progression-free survival

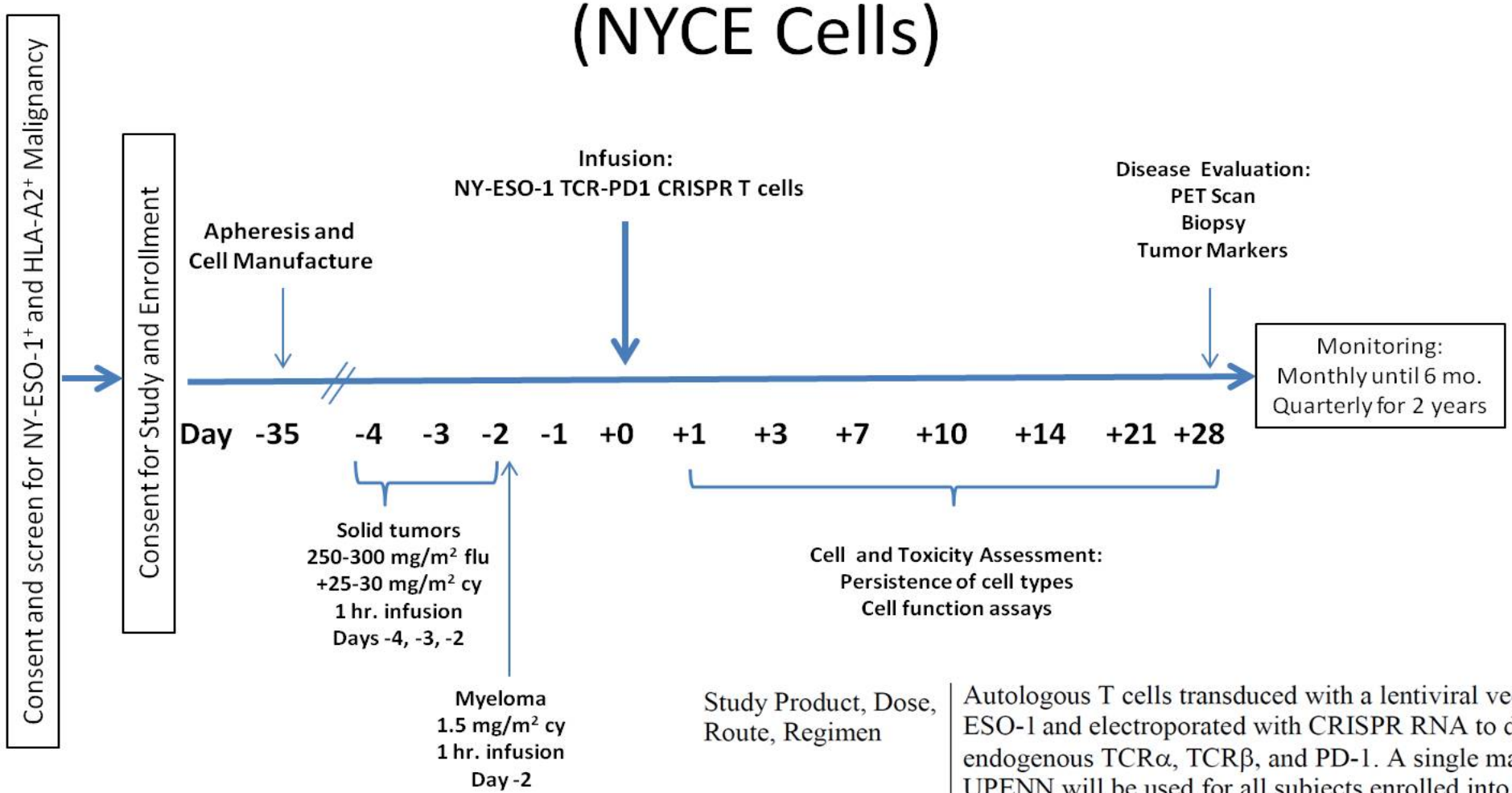
(Rapoport Nat Med 2015, Stadtmauer Blood Adv 2019)

Multiplexed genetic engineering of autologous T cells expressing NY-ESO-1 TCR and CRISPR/Cas9 gene edited to eliminate endogenous TCR and PD-1 (PENN, TMUNITY, PARKER)

- **Overall Rationale:**
  - Increase safety and efficacy by increasing engineered TCR expression and checkpoint inhibition
- **Rationale for endogenous TCRα (TRAC) and TCRβ (TRBC) genes editing:**
  - Reduce endogenous TCR mispairing with exogenous NY-ESO-1 TCR thereby reducing risk of auto-reactivity enhancing recombinant NY-ESO-1 TCR expression on the cell surface for improved potency
- **Rationale PDCD1 gene editing (generate checkpoint resistant T cells)**
  - Gain resistance to PD1 induced suppression thereby improve potency, delay T cell exhaustion



NY-ESO-1 CRISPR (TCR-PD1) Triple Edited T Cell Study Schema (NYCE Cells)



Study Product, Dose, Route, Regimen	Autologous T cells transduced with a lentiviral vector to express NY-ESO-1 and electroporated with CRISPR RNA to disrupt expression of endogenous TCRα, TCRβ, and PD-1. A single manufacturing site at UPENN will be used for all subjects enrolled into this study. A single dose of $1x10^8$cells/kg will be given i.v.

IND 17297 and Clinicaltrials.gov NCT03399448

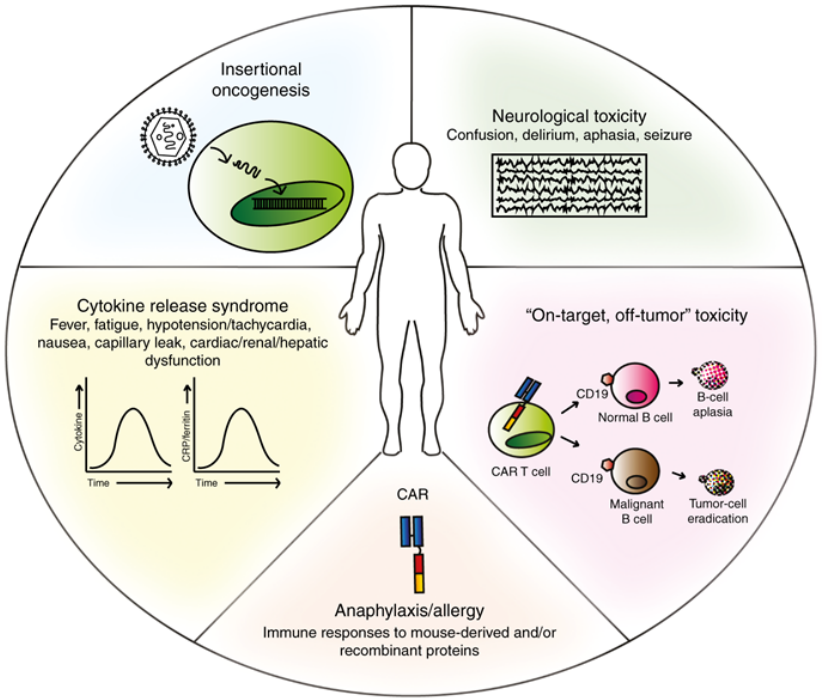
Sponsor: Tmunity and Parker Institute for Cancer Immunotherapy

Toxicities

Associated with CAR T therapy

But not without toxicity

- On target toxicities:
  - Tumor lysis syndrome
  - B cell aplasia
  - Hypogammaglobulinemia
- Off target toxicities:
  - Cytokine release syndrome*
    - persistent high fevers, rigors,
    - myalgias, hypotension, hypoxia,
    - neurologic dysfunction,macrophage activation syndrome
    - very high IL6, also IFN-gamma, TNF
    - responds to steroids → but lose CAR T cells
    - tocilizumab (anti-IL6 receptor mAb) can abrogate CRS
  - CNS toxicity*
    - The causative pathophysiology of these neurologic side effects is unknown, though given similar events reported with blinatumomab administration
    - The neurologic toxicity has been reversible in a majority of cases



Bonifant et al, Molecular Therapy — Oncolytics (2016) 3, 16011

*Potential Life threatening toxicities

Cytokine Release Syndrome (CRS)

- Correlates with:
  - CAR-T activation and expansion
  - Dramatic cytokine elevations (very high levels of **IL6**, IL10, IFNɣ, CRP, ferritin)
  - Many responding patients developed a CRS

- Clinical syndrome:
  - Onset: 1-14 days after infusion
  - Duration: 1-10 days
  - Monitor: VS, ferritin level, and CRP level
  - Fevers come first and get very high (105° F/41° C)
  - Myalgias, fatigue, anorexia
  - Capillary leak, hypoxia and hypotension
    - May require ICU support
  - Altered mental status, seizures, DIC

- Self-limited or anti-cytokine intervention

CRS After CAR T Cells: Risk Factors

Disease Characteristics

- Disease Burden (ALL)[1-4]

Therapeutic Characteristics

- Infusional Dose[3,4,6]
- Product variance
- LD chemotherapy[4]

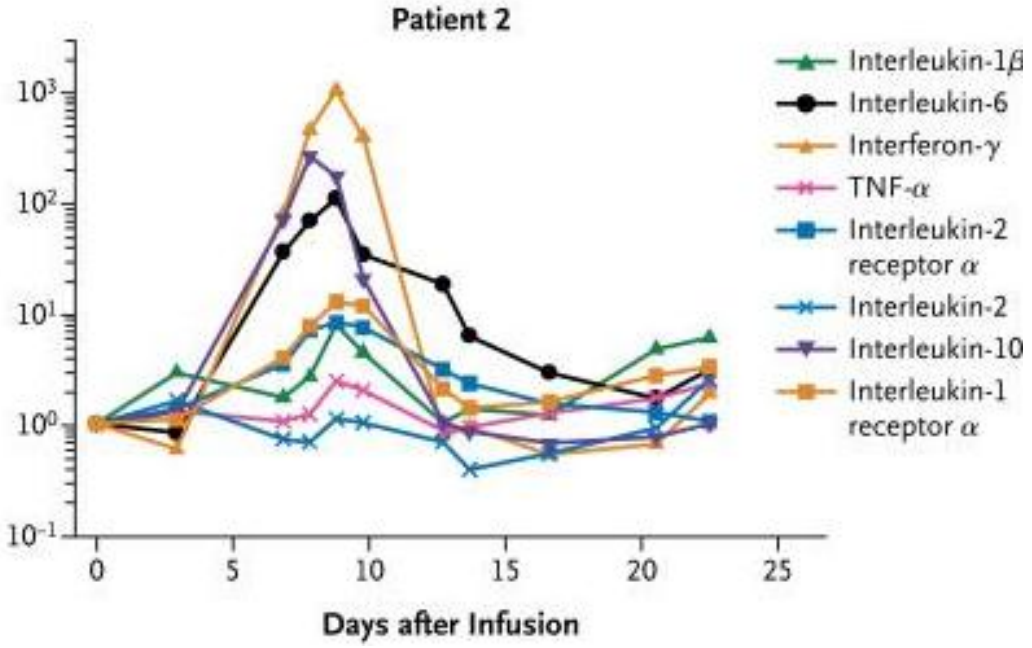
Correlates with Severe Course

- Cytokines and CRP[1,5]
- Concurrent infectious illness[6]

[1]Maude et al. NEJM 2014
[2]Davila et al. SciTranMed 2014
[3]Lee et al. TheLancet 2015
[4]Turtle et al. JCI 2016
[5]Teachey et al. CancerDisc. 2016
[6]Frey et al. ASCO 2016

CRS: Cytokine Profiles

- Clinical Laboratory Correlates:
  - Ferritin and CRP
- Investigational Correlates: Direct Impact on Care[1]!
  - Cytokine Profiles: IFNɣ. **IL6**. IL2R. IL10



[1]Grupp et al. NEJM 2013

CRS After CAR T Cells: Anti-cytokine Management

CRS with high IL6

Tocilizumab for CRS[1]:

- Humanized monoclonal antibody to IL6-R
- FDA approved adult RA, JIA
- Limited inherent toxicity
- Adopted by most programs
- Effective for most patients

[1]Grupp et al. NEJM 2013

“The Antidote”: Tocilizumab

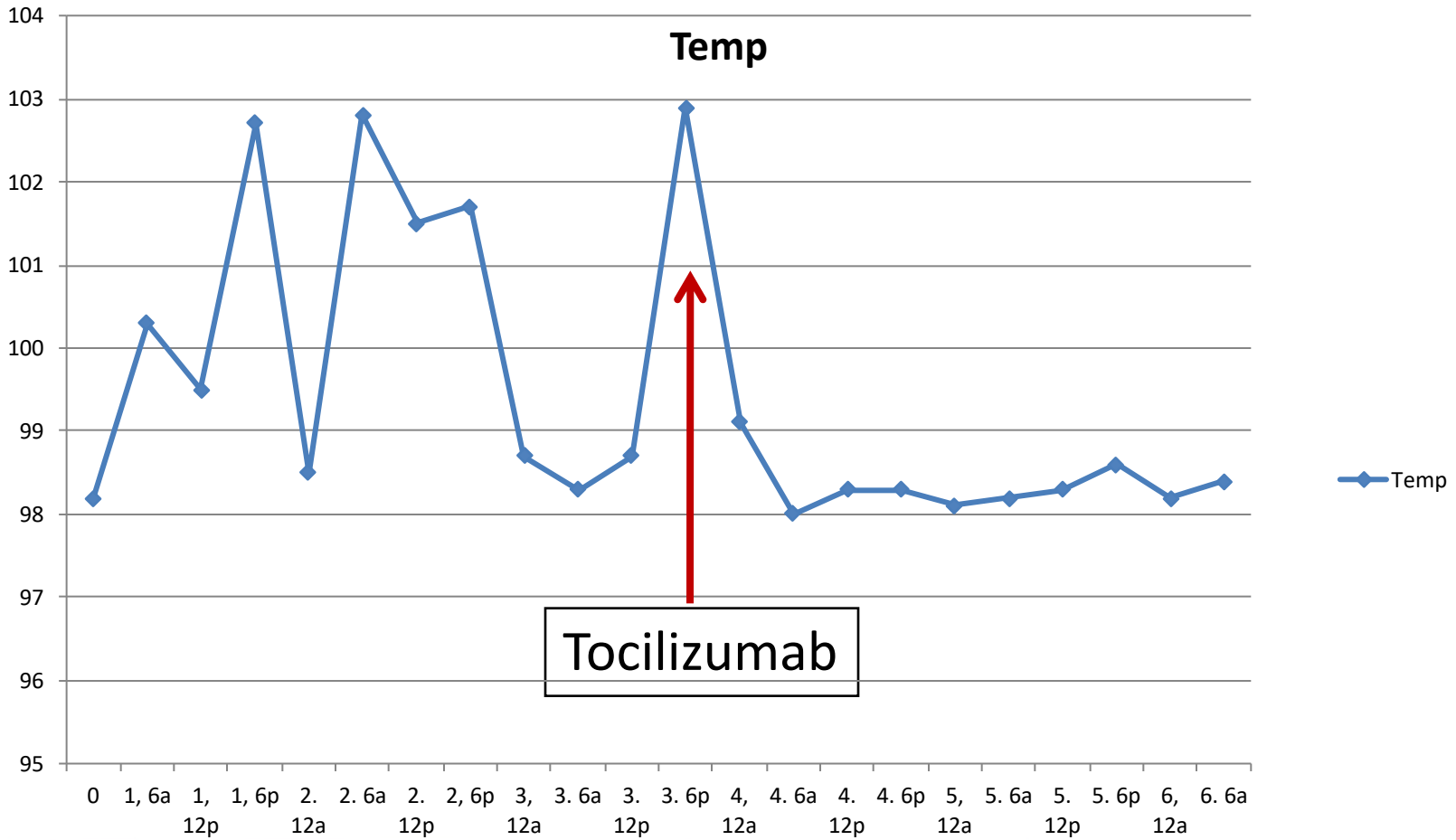
- Humanized monoclonal antibody to IL-6
- Can rapidly reverse CRS[1]
- Ensure that 2 doses of tocilizumab are available prior to infusion of CAR-T cells
- Monitor patients closely at least daily for 7 days following infusion for signs and symptoms of CRS
- May be admitted for this close observation then closely as out patient for 4 weeks following the CAR T infusion.
- Counsel patients to seek immediate medical attention should signs or symptoms of CRS occur at any time
- At the first sign of CRS, institute treatment with supportive care, tocilizumab or tocilizumab and corticosteroids as indicated

1. Tocilizumab PI. 2017. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125276s114lbl.pdf. 2. Le RQ et al. *Oncologist*. 2018;23(8):943-947.

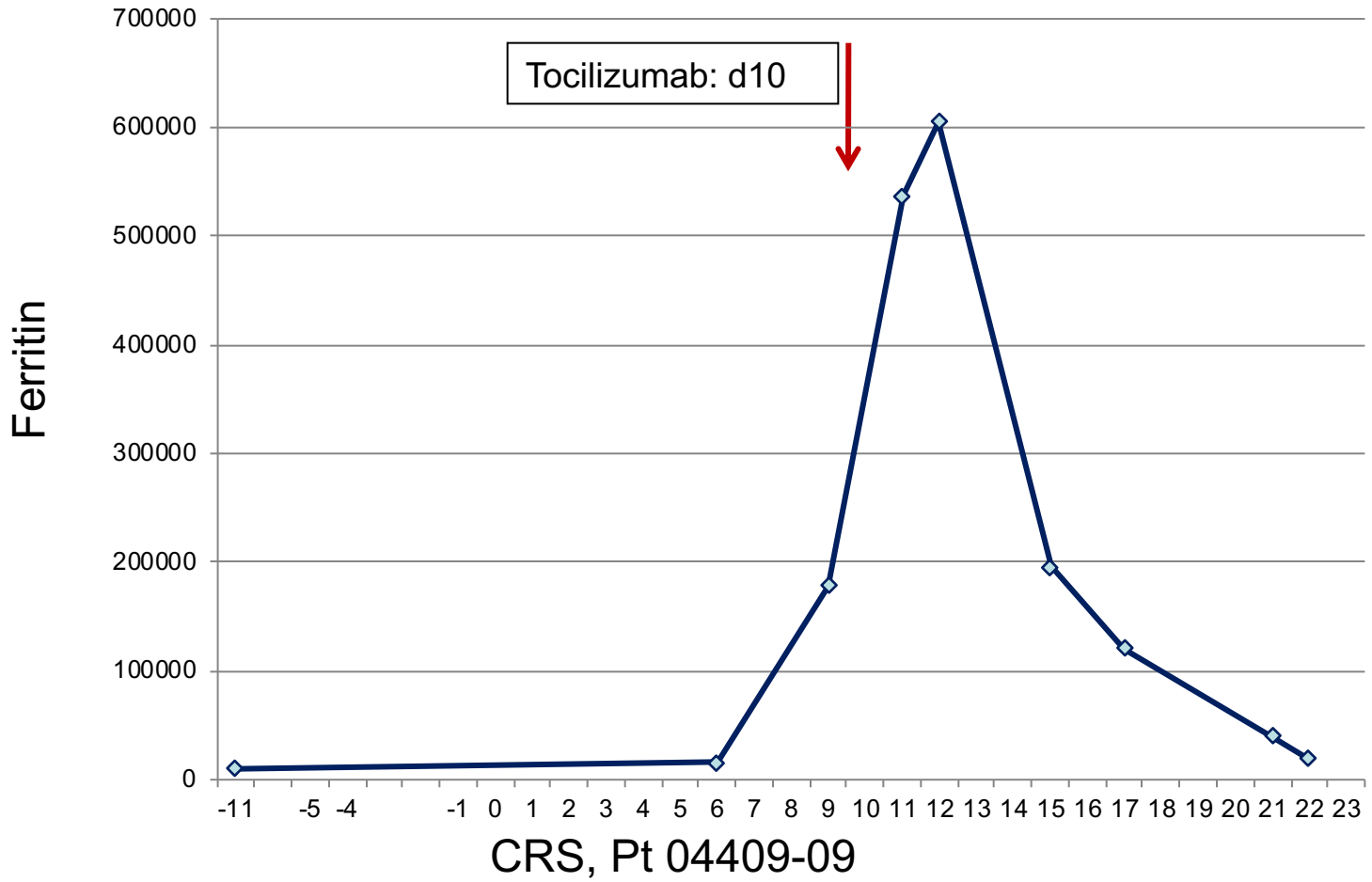
CRS With CART19 Therapy

Ref	Program/ CAR	Population	Response	CRS
Acute Lymphoblastic Leukemia				
Maude et al. NEJM 2014	PENN 4-1BB	N=30(ALL) Peds&Adults	CR=90%	100% CRS 27% Severe
Davila et al. SciTrMed 2014	MSK CD28	N=16 (ALL) Adults	CR=88%	43% Severe
Lee et al. Lancet 2015	NCI CD28	N=21 (ALL) Peds&AYA	CR=67% Intent to Treat	76% CRS 28% Severe
Turtle et al. JCI 2016	Seattle 4-1BB	N=30 Adults	CR=93%	83%CRS
Non-Hodgkins Lymphoma & Chronic Lymphocytic Leukemia				
Kochenderfer JCO 2015	NCI CD28	N=15 (NHL/CLL)	CR=53% PR=27%	27% Severe
Porter et al. SciTrMed2014	PENN 4-1BB	N=14(CLL)	CR=29% PR=29%	42% Severe

CRS: Clinical Response to Tocilizumab



CRS: Ferritin Response to Tocilizumab



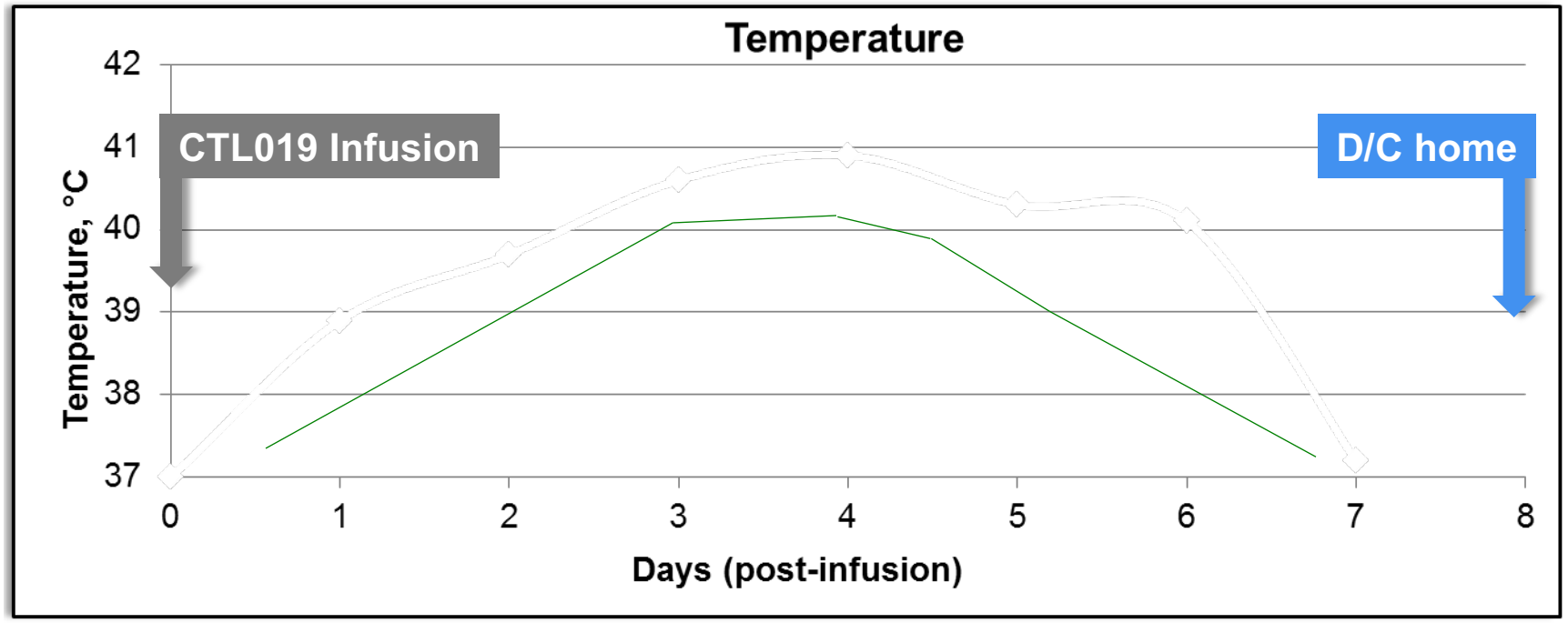
Mild CRS: Case #1

NHL History

- 59 yo male
- R CHOP x 6 cycles-> CR
- Relapsed 5 mo later
- Salvage with R-ICE x 2 cycles followed by AutoBMT
- Relapsed 3 mo later by radiographic PD

Timing	Key events
Month-3	Re-induction with R ICE (response)
Month -2	T cells collected
Week -1	lymphodepleting chemotherapy (fludarabine/cyclophosphamide)
Day -1	**PET/CT with PR** BM blasts, no peripheral blasts

Mild CRS: Case #1



Anti-pyretics (days 3-6)

Myalgias (days 2-7)

Antibiotics (days 1-7)

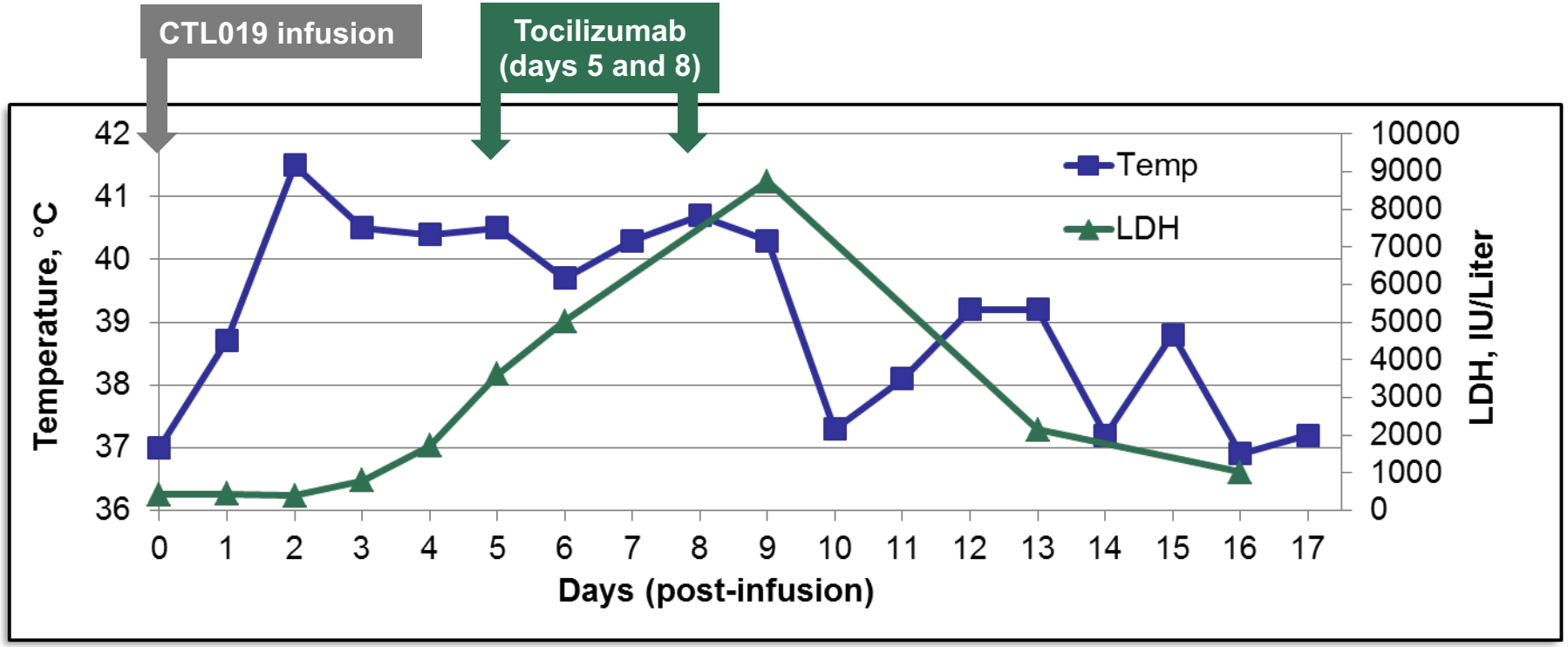
Severe CRS: Case #2

ALL History

- 22 yo male ALL
- 1st relapse in maintenance therapy
- Refractory to reinduction

Timing	Key notes
Month -2	T cells collected after failed re-induction
Month -1	Started hydroxyurea
Week -1	lymphodepleting chemotherapy (fludarabine/cyclophosphamide)
Day -1	**97%** BM blasts , no peripheral blasts

Severe CRS: Case #2



Rasburicase (Day 9)

Confusion (day 2-11)

High dose steroids (days 7-11)

Respiratory support (days 3-10)

High-dose vasopressors (days 5-9)

Transfusion support (days 2-15) FFP (days 2 and 8) Cryoprecipitate (days 10-15)

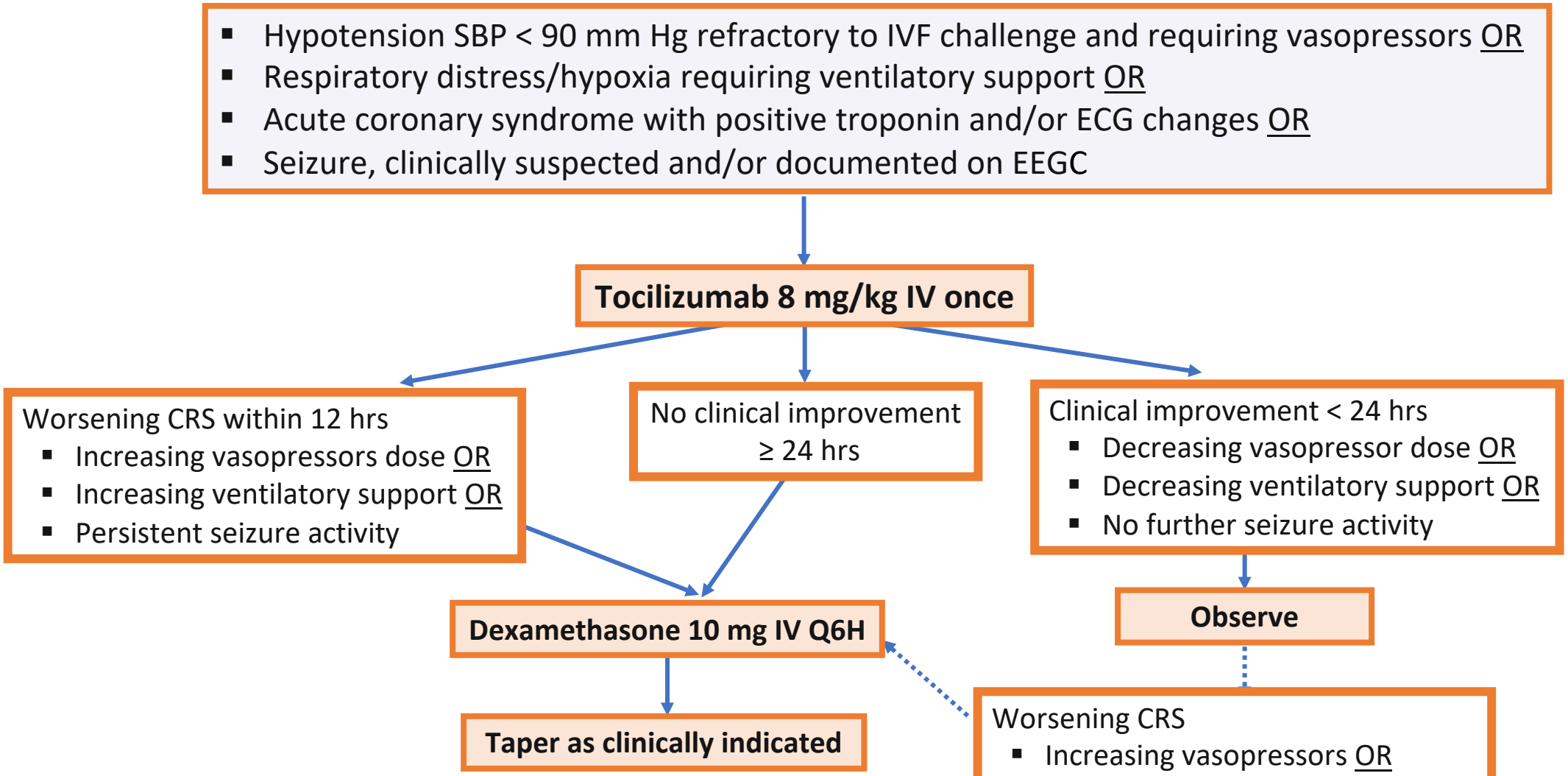
ASBMT Consensus Grading for CRS Associated with Immune Effector Cells (IEC)

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	$T_m \geq 100.4^oF$	$T_m \geq 100.4^oF$	$T_m \geq 100.4^oF$	$T_m \geq 100.4^oF$
With either:				
Hypotension	None	Responsive to fluids	Requiring 1 vasopressor (w/ or w/o vasopressin)	Requiring multiple vasopressors (excluding vasopressin)
And/or				
Hypoxia	None	Low-flow nasal cannula or blow-by	High-flow nasal cannula, face mask, non-rebreather mask, or Venturi mask	Requiring positive pressure (CPAP, BiPAP Intubation and mechanical ventilation)

- Organ toxicities associated with CRS may be graded according to CTCAE v5.0, but they do not influence CRS grading
- Low-flow nasal cannula: O2 delivered at <6 L/minute.

- Lee DW, et al. Biol Blood Marrow Transplant. 2018 Dec 25. pii: S1083-8791(18)31691-4.

CRS Management



Park. ASCO 2016. Abstr 7003

CAR T cells for ALL:
Optimizing Risk: Benefit Ratio

- Delivery of CAR T cells:[3]
  - Dose adjustment based on disease burden
  - Fractionated dosing: Real time dose modification by CRS symptoms
- CAR T modifications:[4,5,6]
  - Create CARTs with targets for destruction:

    (CD20, EGFR, HSV thymidine kinase, caspase 9)
  - "On switch": additional signal (drug) to be activated

[1]Gardner et al. ASH2016-586
[2]NCT02906371(CHOP)
[3]Frey et al. ASCO. 2016
[4]DiStasi et al, NEJM. 2011
[5]Casucci et al, Molecular Therapy. 2013
[6]Wu et al, Science. 2015

Neurotoxicity

Second Most Common Toxicity Associated with CAR T-cell Therapy

- Range of Symptoms

  diminished attention, language disturbance, confusion, disorientation, agitation, aphasia, tremors, seizures, encephalopathy
- Pathophysiology
  - Unclear; however is likely related to T-cell
  - Passive diffusion of cytokines
  - Expansion of CAR T-cells into CNS
- Predictors
  - High Disease Burden
  - High IL6 on Day1[5]

- Neurotoxicity and CRS follow a different course of onset and resolution
- Onset varies and can be biphasic:
  - *Early* – Symptoms occur concurrently with CRS symptoms (~within first 5 days)
  - *Late* – Begins after CRS symptoms have resolved
  - *Delayed* – Most neurotoxicity events (88-98%) occur within 8 weeks after cell infusion (seizures, episodes of confusion)

1Maude et al. NEJM 2014 2Davila et al. SciTranMed 2014 3Lee et al. TheLancet 2015 4Kochendorfet al. JCO 2015 [5]Turtle et al. JCI. 2016

Immune Effector Cell-Associated Encephalopathy (ICE) Score

ICE Score
How many of the following is the patient oriented to: year, month, city, hospital
Identify 3 objects. How many can the patient name?
Can follow commands
Can write a standard sentence
Can count backwards from 100 by 10

Score 10: No impairment
Score 7-9: Grade 1
Score 3-6: Grade 2
Score 0-2: Grade 3

***Combine with other ICANS assessments for final grade**

Lee DW, et al. Biol Blood Marrow Transplant. 2018 Dec 25. pii: S1083-8791(18)31691-4.

Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE SCORE	7-9	3-6	0-2	0 *(patient is unarousable and unable to perform ICE)*
Depressed LOC attributed to no other cause	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is **unarousable** or requires vigorous or repetitive tactile stimuli to arouse. **Stupor or coma**
Seizure	N/A	N/A	**Any clinical seizure** focal or generalized that resolves rapidly; or Non-convulsive seizures on EEG that resolve with intervention	**Life-threatening prolonged seizure** (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor findings	N/A	N/A	N/A	**Deep focal motor weakness** such as hemiparesis or paraparesis
Raised ICP / Cerebral edema	N/A	N/A	Focal/local edema on neuroimaging	**Diffuse cerebral edema** on neuroimaging; Decerebrate or decorticate posturing; or Cranial nerve VI palsy; or Papilledema; or Cushing's triad

Managing Neurotoxicity of CAR T-Cell Therapy

- **Tocilizumab** might reverse neurotoxicity during first phase but not second phase
- **Corticosteroids** may be used to manage neurotoxicity if tocilizumab is not effective[1]
- **Seizure prophylaxis**

1. Brudno. Blood. 2016;127:3321. 2. Santomasso. Cancer Discov. 2018;8:958.
3. Neelapu. Nat Rev Clin Oncol. 2018;15:47. 4. Tisagenlecleucel PI.

Management of CRES

CAR-Related Encephalopathy Syndrome

- **Grade 1/2**
  - Requires vigilant supportive care
  - Neuro consult with diagnostic imaging
  - Daily monitoring with EEGs
  - Consider tocilizumab
  - **Grade 2:** tocilizumab/siltuximab or high-dose corticosteroids and consider ICU transfer
- **Grade 3/4**
  - Vigilant supportive care and neuro workup
  - ICU transfer
  - Consider tocilizumab/siltuximab
  - Corticosteroid taper for worsening
- **Grade 4:** ICU monitoring and consider mechanical ventilation
  - Anakinra (IL1 inhibitor)

Adapted from MD Anderson Cancer Center. Chimeric Antigen Receptor (CAR) Cell Therapy Toxicity Assessment and Management - Adult.

Neurotoxicity of CART19 Therapy

Ref	Program CAR	Population	Response	CRS	Neuro Toxicity
Acute Lymphoblastic Leukemia					
Maude et al. NEJM 2014	PENN 4-1BB	N=30(ALL) Peds&Adults	CR=90%	100% CRS 27% Severe	43% Total Encephalopathy Aphasia Seizure (1)
Davila et al. SciTrMed 2014	MSK CD28	N=16 (ALL) Adults	CR=88%	43% Severe	25% Gr3-4 Encephalopathy Seizure
Lee et al. Lancet 2015	NCI CD28	N=21 (ALL) Peds&AYA	CR=67% Intent to Treat	76% CRS 28% Severe	29% Total hallucinations Dysphasia encephalopathy
Turtle et al. JCI 2016	Seattle 4-1BB	N=30 Adults	CR=93%	83%CRS	50% Severe
Non-Hodgkins Lymphoma & Chronic Lymphocytic Leukemia					
Kochenderfe JCO 2015	NCI CD28	N=15 (NHL/CLL)	CR=53% PR=27%	27% Severe	40% Total Encephalopathy Aphasia, R facial par
Porter et al. SciTrM2015	PENN 4-1BB	N=14(CLL)	CR=29% PR=29%	42% Severe	43% Total 1/14 Grade 4

Toxicities in BCMA Trials for Myeloma

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%
Janssen[4]	57	76%	7%	42%	2%	

[1]Ali, Blood 2016 and Brudno, J Clin Oncol 2018; [2]Cohen, JCI 2019 [3]Raje, NEJM 2019 ; [4]Zhao. ASH 2018

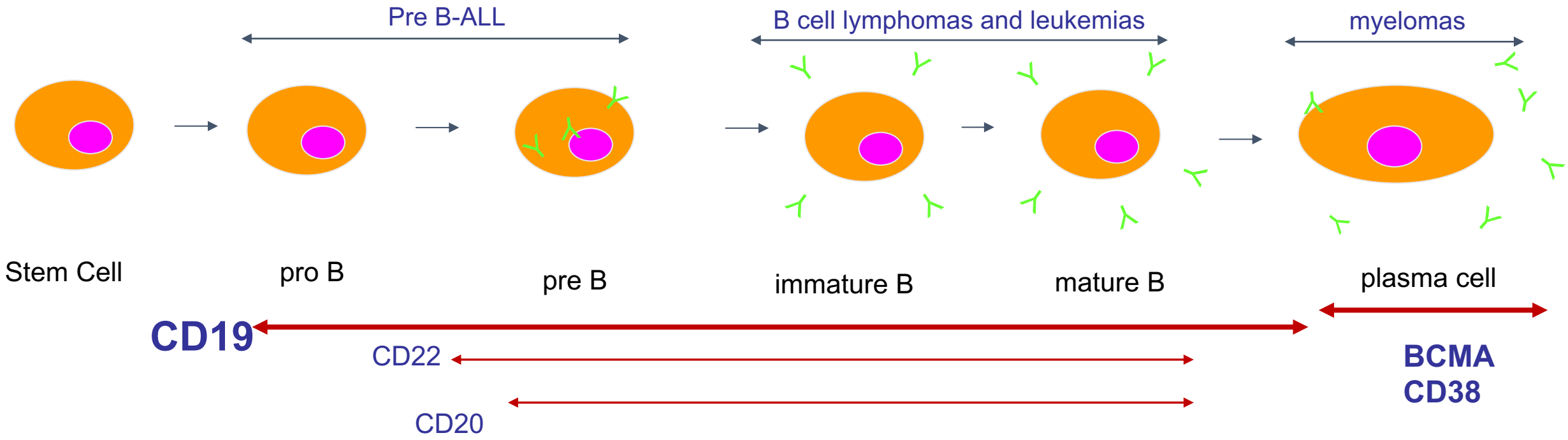
Premedication and Prophylaxis Considerations

- Cell-infusion pre-medications: acetaminophen and diphenhydramine Use of uric acid lowering medications to prevent TLC
- No steroids from the start of lymphodepleting chemotherapy ??
- Infection prophylaxis
  - Antiviral
  - Antifungal and fluoroquinolone during neutropenic period
  - PJP prophylaxis
- Seizure prophylaxis
  - Examples: levetiracetam 500-750 mg PO BID day -1/0 to day 30

Neelapu SS, et al. Nat Rev Clin Oncol. 2018; 124:188-195.

B-cell Aplasia and Hypogammaglobulinemia

- **On target expected SE is B cell aplasia**
- **Correlates with CART persistence**
- **Successfully managed with IVIG replacement**
- **No excessive or frequent infections**



1,Scheuermann RH, et al. *Leuk Lymphoma*. 1995;18:385-397. 2. Image adapted from Janeway CA, Travers P, Walport M, et al. *Immunobiology*. 5th ed. New York, NY: Garland Science; 2001:221-293.

3. Scheuermann RH, et al. *Leuk Lymphoma*. 1995;18:385-397.

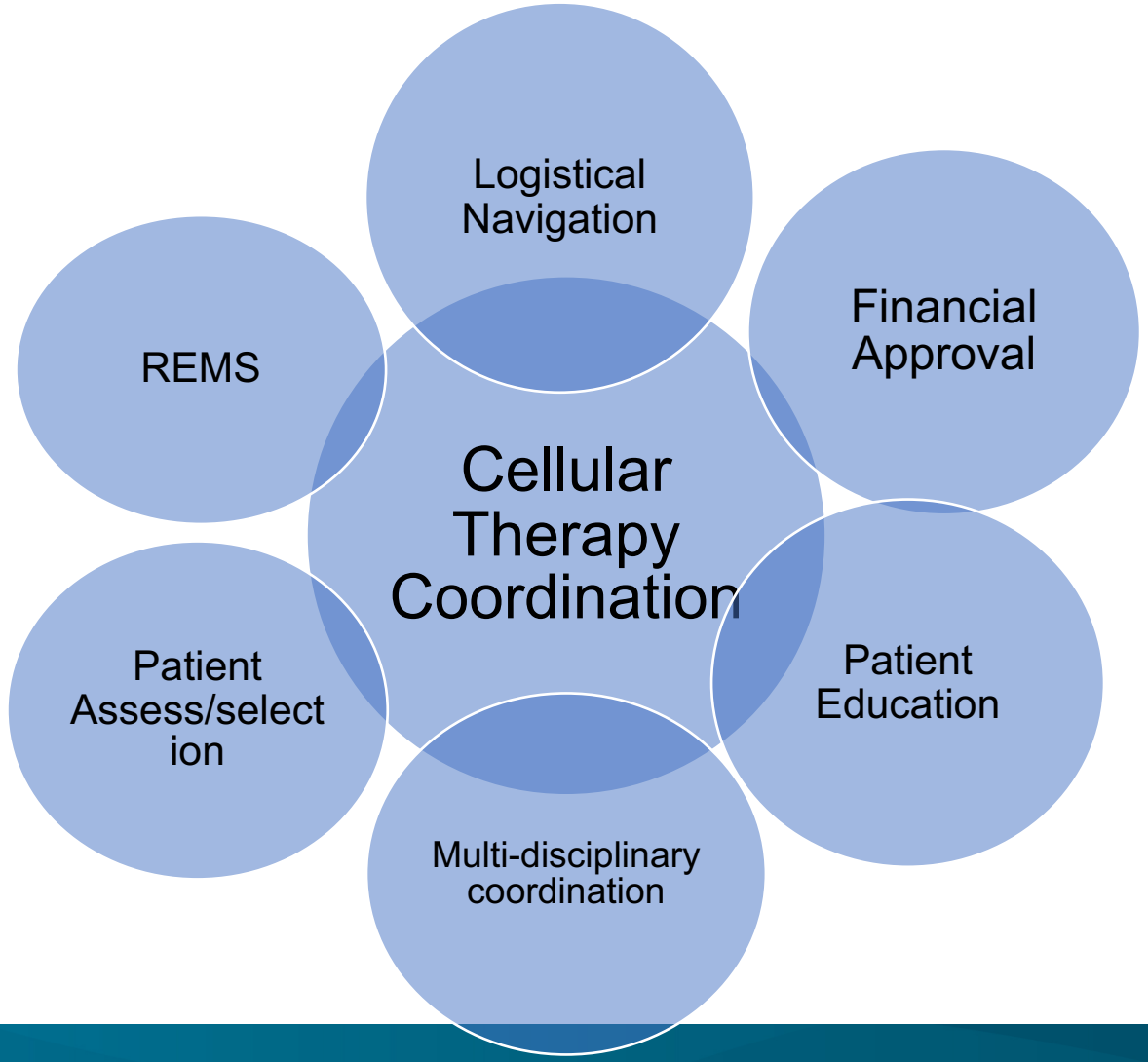
4. Feldman M, Marini JC. Cell cooperation in the antibody response. In: Roitt I, Brostoff J, Male D, eds. *Immunology*. 6th ed. Maryland Heights, Missouri: Mosby;2001:131-146.

Additional Toxicities Associated with CAR T-cells

- Tumor lysis syndrome
  - Use of uric acid lowering meds with high burden of disease
- Infections (opportunistic)
  - IVIg
  - Antiviral, Antibacterial, Antifungal
- Prolonged cytopenias
  - Continued monitoring of CBC
  - Growth factor as needed

Bonifant CL, et al. Oncolytics. 2016;3:16011.
Brudno JN, et al. Blood. 2016;127:3321-30. Neelapu SS, et al. Nat Rev Clin Oncol. 2018; 124:188-195.

Cellular Therapy Coordination



What's Next in Cellular Immunotherapy ?

- Constructs
  - Antigen recognition
  - Stimulatory molecules
- Vectors
  - Viral
  - Non-viral approaches
- Dose
- Off switches
  - Suicide genes/safety domains
- Lympho-depletion
- Single vs serial infusions
- Patient selection
  - Test for target
  - Early vs heavily pretreated
- Toxicities
  - Timing of tocilizumab

- Gene editing
  - "Universal" or "Off the Shelf" CAR T cells
  - CRISPR gene edited NY-ESO1 TCR T cells
- Dual CARs
- Combinations with
  - IMIDs
  - Checkpoint inhibitors
- Use in other cancers

[1]*Grupp et al. ASHAbst221*
[2]*Chang et al ASH Abst 587*
[3]*Shah et al*: ASHAbst 650
[4]*Neelapu et al*. LBAbst 6

Clinical Pearls

- CAR T therapy is an effective form of cellular immunotherapy for ALL, NHL and multiple myeloma.
- It is multi-step process and requires great deal of coordination of care.
- There are unique toxicities associated with this therapy, which vary by product and disease being treated.
- We now are more comfortable with earlier intervention without loss of effectiveness or persistence of these cells
- This is just the beginning of adoptive immunotherapy!!
  - For use in other malignancies; with less toxicities and more persistence and availability.

More Questions?

Come see us in the Skybridge Lobby near Registration from 8:15 to 8:45 am tomorrow.

SMARTIE

This has been a SMARTIE presentation.

To access your post-session questions, you can:

- Click on the link that was sent to you via email
- Visit the SMARTIE station
- Go to jadprolive.com/smartie2019