UPDATE ON ENGINEERED CELL THERAPIES: CAR-T AND BEYOND

Stephan Grupp, MD, PhD

Director, Susan S. and Stephan P. Kelly Center for Cancer Immunotherapy

Children's Hospital of Philadelphia®
Cellular Therapy & Transplant

Disclosures

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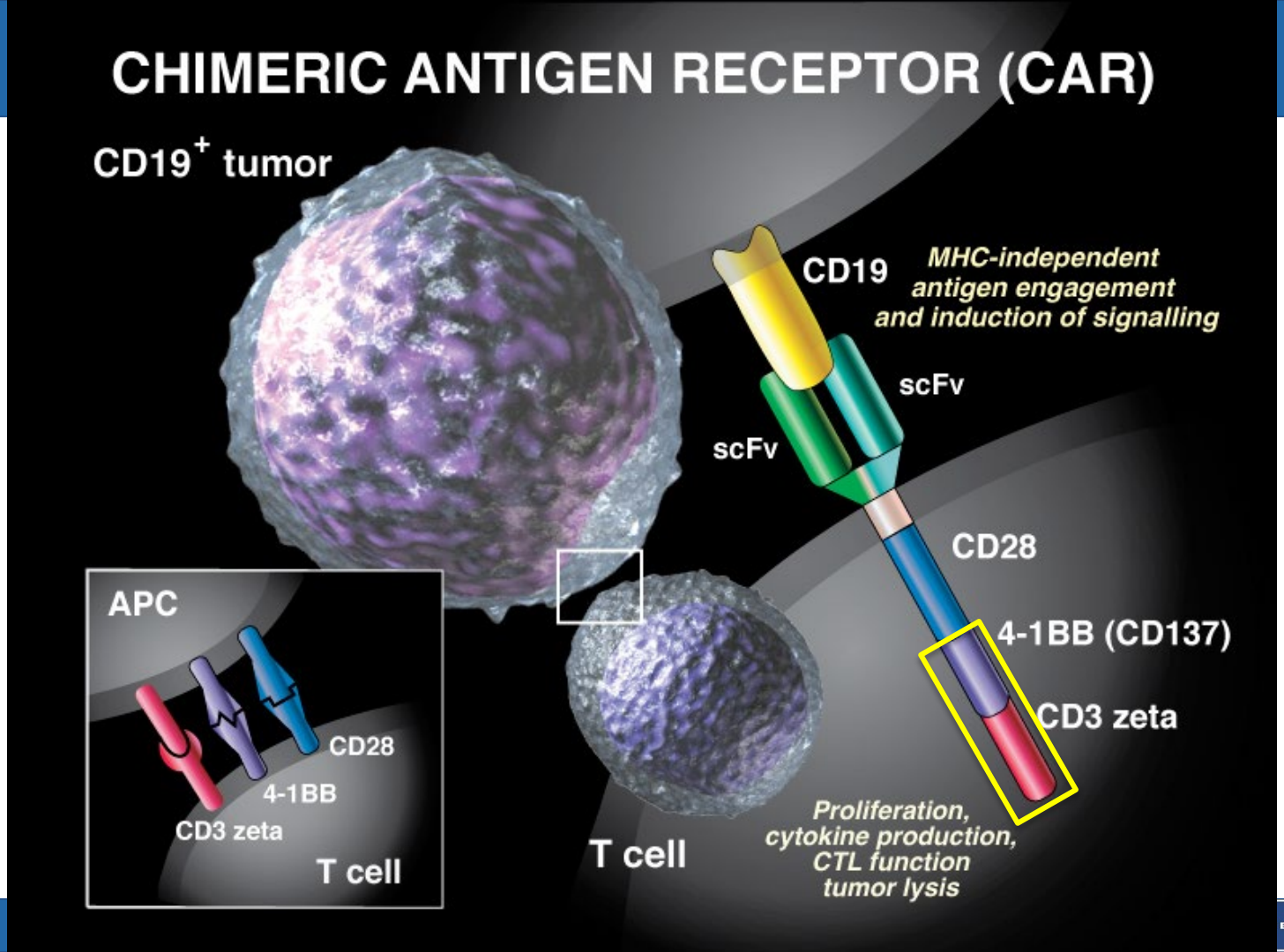
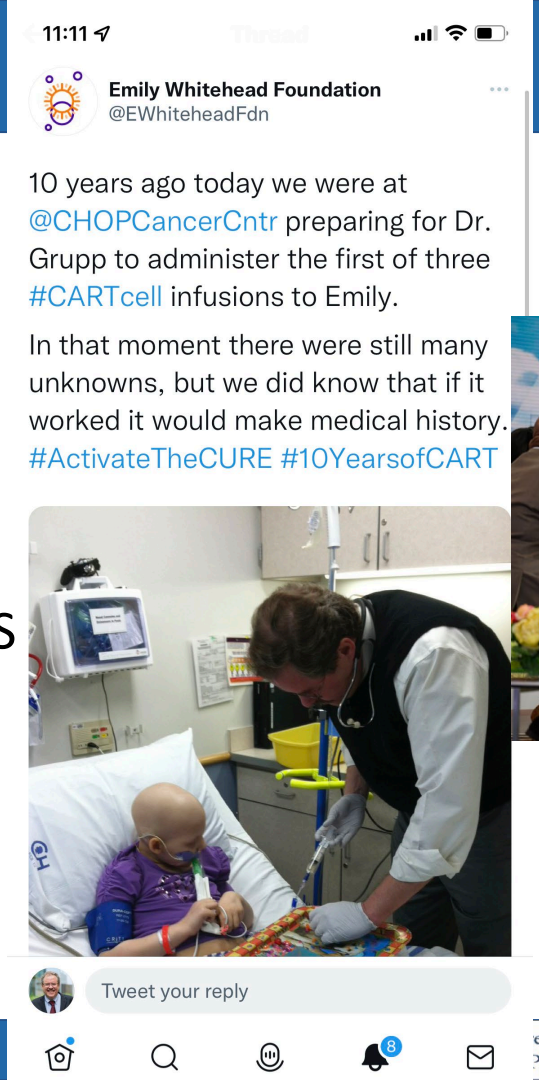
- Study steering committees, consulting, or scientific advisory boards: Novartis, Allogene, Adaptimmune, TCR2, Cabaletta, Juno/BMS, CBMG, GlaxoSmithKline, Cellectis, Vertex, J&J/Janssen, Roche
- Toxicity management patent managed by U Penn policies


CHIMERIC ANTIGEN RECEPTOR (CAR)
CD19+ tumor
CD19
MHC-independent antigen engagement and induction of signalling
scFv
scFv
CD28
4-1BB (CD137)
CD3 zeta
APC
CD28
4-1BB
CD3 zeta
T cell
T cell
Proliferation, cytokine production, CTL function tumor lysis

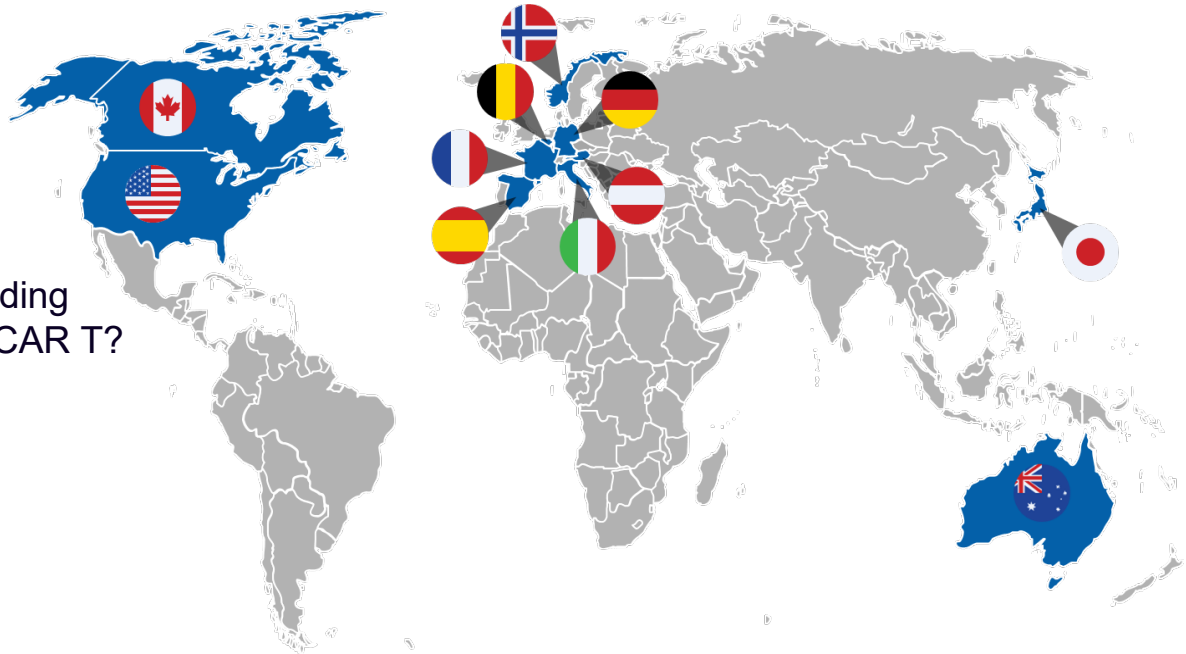
Journey to CAR T approval

- 1st ALL patient treated in 2012
- Global registration trial began 2015
- ODAC July 2017
- FDA approval 8/30/17
- 1st CAR T approved
- 1st gene therapy approved in US
- 2022 cell and gene:
  - 6 CAR T (all heme malignancies)
  - 2 infused gene therapies (non cancer)



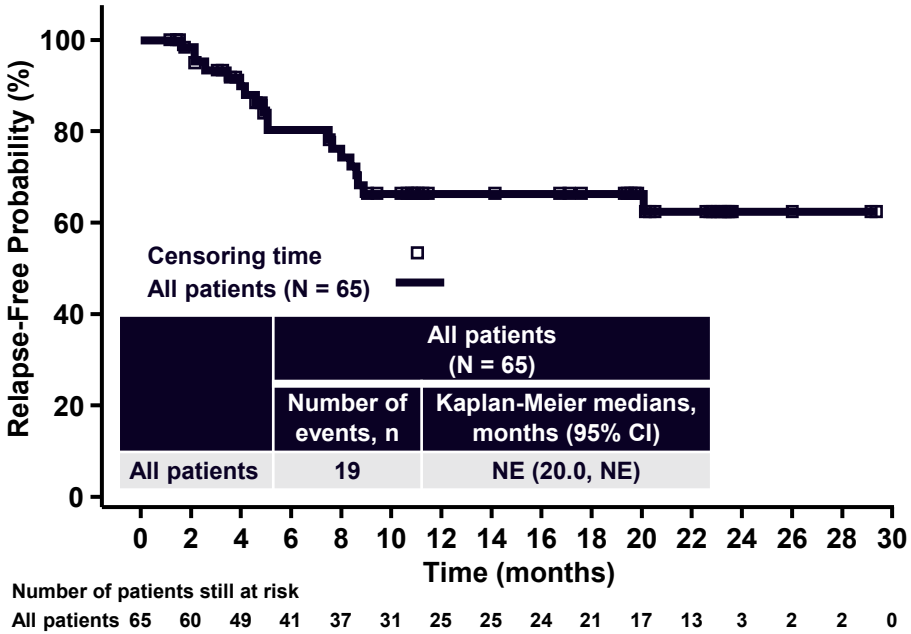
Global, multicenter trial
ALL registration study ELIANA

- ELIANA is a single arm global study with centralized manufacturing of tisagenlecleucel
- 25 sites in 11 countries across North America, Europe, Australia, and Asia
- Can we replicate the single arm trial?
- Could we see similar remission induction (~90%)
- Similar safety in outstanding centers that are new to CAR T?



FPFV=8 APR 2015
Data cutoff: 23 NOV 2016

High Response Rate;
Median Duration of Remission Not Reached

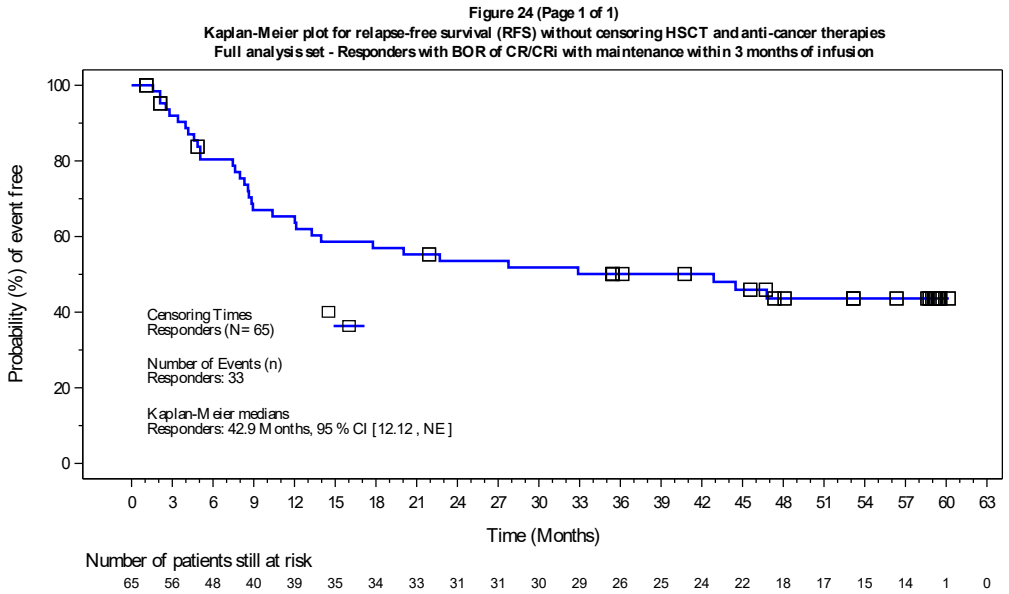


- CR + CRi (within 3 months) 82% (65/79 infused; 95% CI, 72-90)[a,b]
- 98% (64/65) MRD(–)
- RFS among responders
  - 12-month: 66% (95% CI, 52-77)
  - 18-month: 66% (95% CI, 52-77)
  - 24-month: 62% (95% CI, 47-75)
- OS among all infused patients
  - 12-month: 76% (95% CI, 65-85)
  - 18-month: 70% (95% CI, 58-79)
  - 24-month: 66% (95% CI, 54-76)

Note: Only patients who achieved CR or CRi were included. Time is relative to onset of remission.
[a] The response was unknown in 6 patients.
[b] While in remission, 8 patients went on to stem-cell transplantation.
[c] MRD negative = MRD < 0.01%, as assessed by flow cytometry.
CR, complete remission; CRi, complete remission with incomplete blood count recovery

Median RFS Was 46.8 Months on ELIANA

RFS for Patients With a CR/CRi within 3 months
5-year RFS: 43.6% (95% CI, 31%-56%)

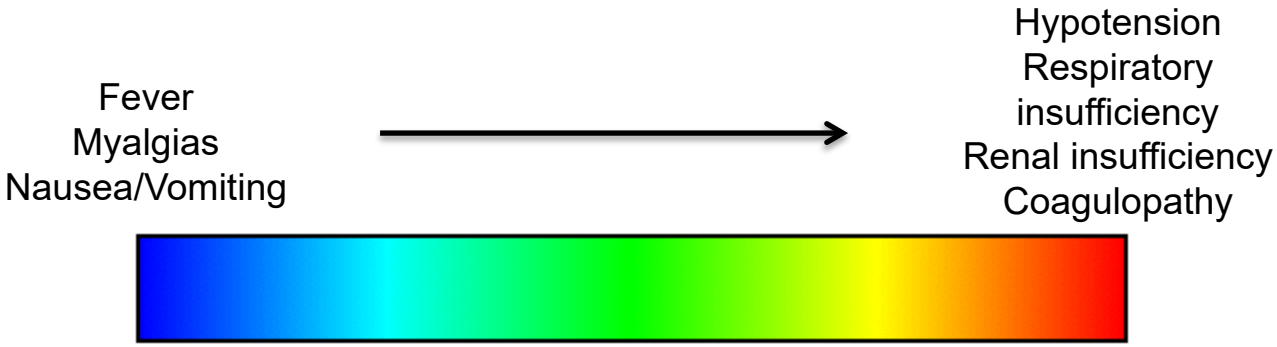


Note: RFS is without censoring for SCT and other cancer therapies
CR, complete remission; CRi, complete remission with incomplete blood count recovery; NE, not estimable; RFS, relapse-free survival.

Cytokine Release Syndrome (CRS)

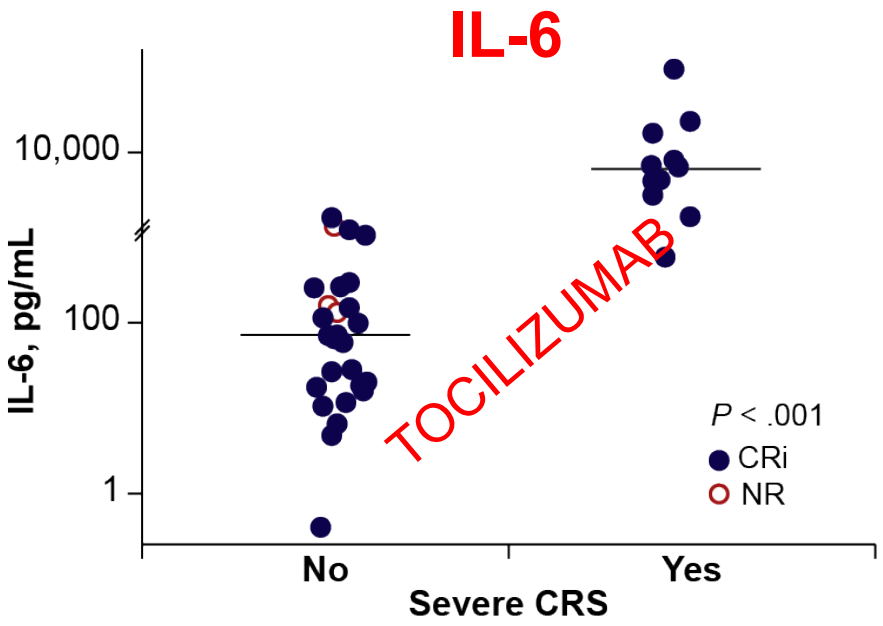
CRS is related to T cell expansion and may be necessary for efficacy

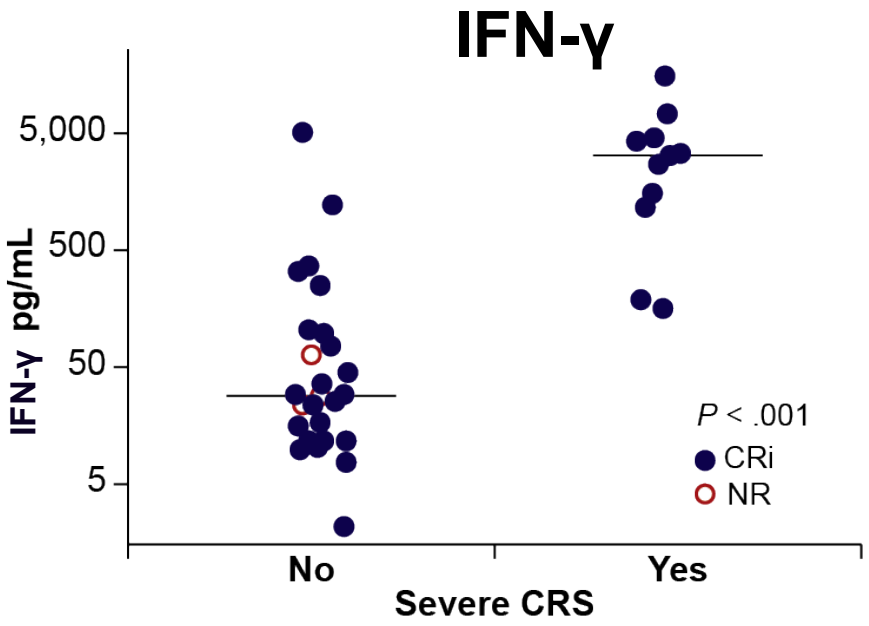
- Symptoms typically occur 1-14 days after CTL019 cell infusion in ALL



- Severity scales with disease burden

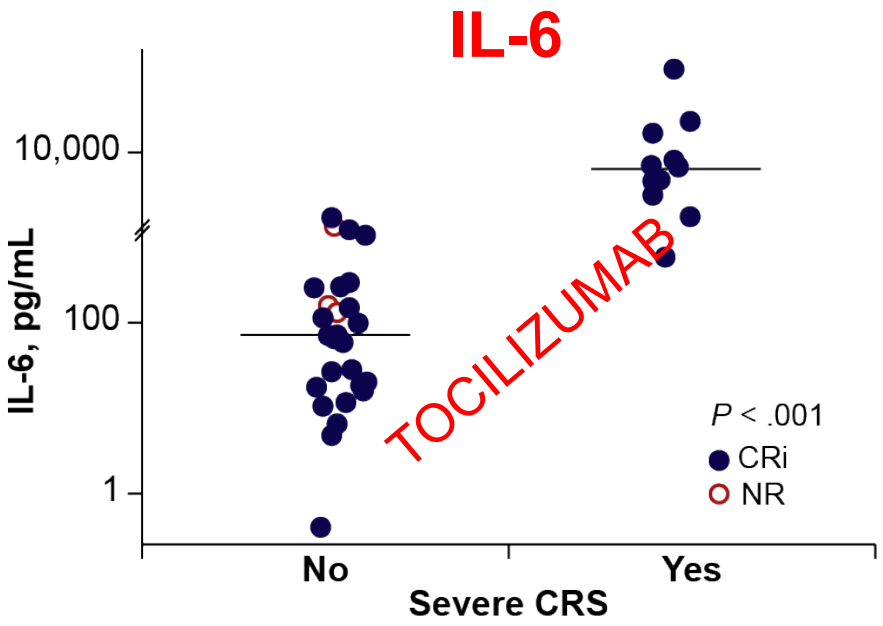
Cytokine Release Syndrome Associated With IFN-γ and IL-6

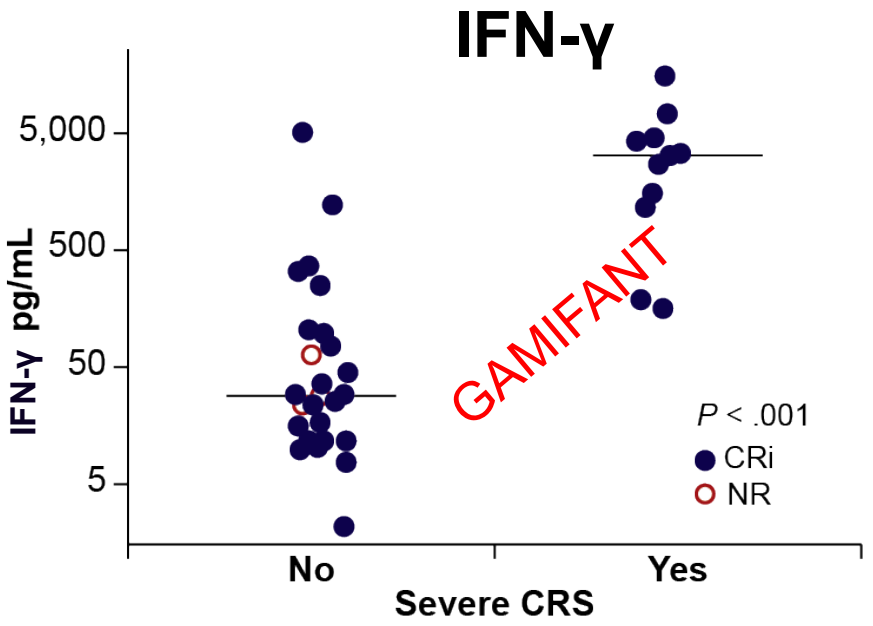




1. Maude SL et al. *N Engl J Med*. 2014;371:1507-1517.

Cytokine Release Syndrome Associated With IFN-γ and IL-6





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

CHOP/PENN CRS MANAGEMENT

- Response based toxicity management (not grading based)
- Step 1 – toci for unstable hypotension (most common) or other significant changes in clinical status
  - Second bolus in a short time – start of unstable hypotension
  - Trigger for toci – rapid decline, escalating single pressor, definitely 2nd pressor. OR – just PICU for one pressor
- Step 2 – no change in 12-18 hrs – methylpred 2 mg/kg or equiv
  - rapid wean of steroids after hypotension resolved

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

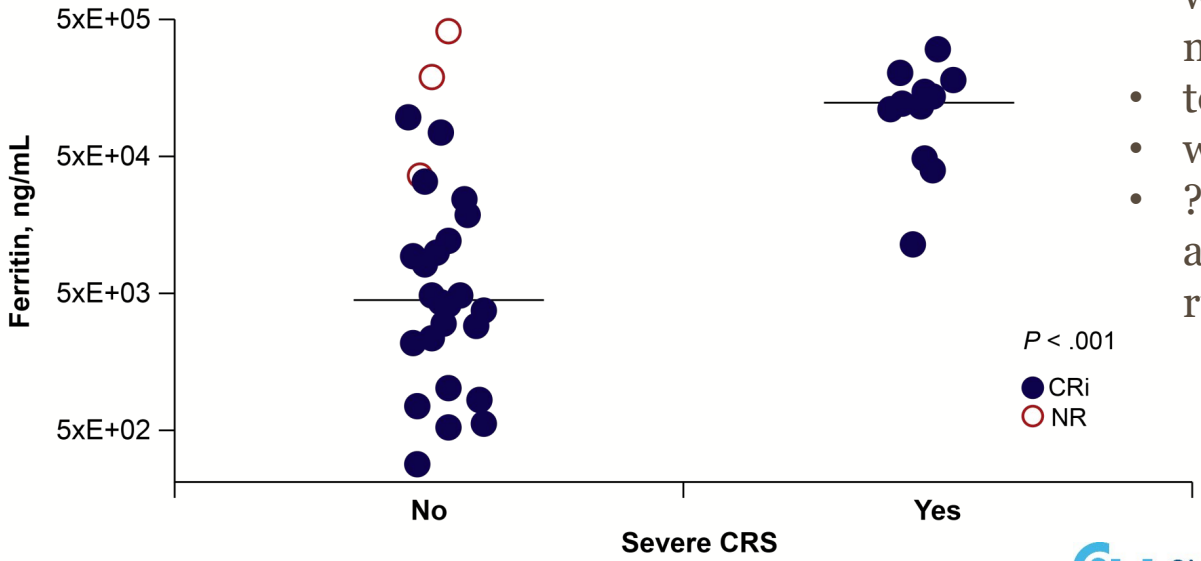
CHOP/PENN CRS MANAGEMENT

- Response based toxicity management (not grading based)
- Step 3 – no improvement in 12-18 hrs – 2nd dose of toci
- Step 4 – no improvement
  - consider siltuximab.

  Alternatives? Maybe Gamifant (IFNg blockade)

SEVERE CRS

High ferritin levels suggest overlap between CRS and Macrophage Activation Syndrome (MAS/HLH)



- CRS (usual timing) **is** MAS
- Similar cytokines
- w/ CART22, there may be **late CRS**
- toci refractory
- w/ or w/o fever
- ?? responds to anakinra or ruxoltininb

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

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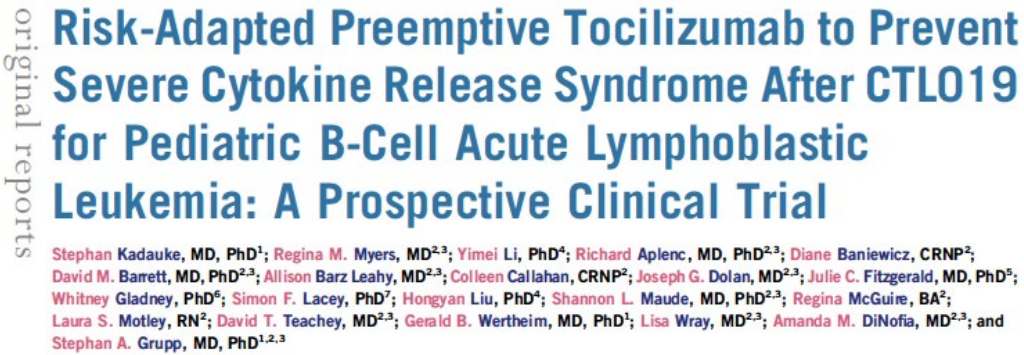
EARLY/PRE-EMPTIVE CRS TREATMENT

- "Early toci" trial testing tocilizumab pre-emptive therapy N=70
- High disease burden (>=40% blasts) only N=15
- Single dose toci given at time of sustained fever (true CRS onset) to pre-empt grade 4 CRS
- Followed by standard CRS management for both high and low tumor burden cohorts

Kadauke et al, JCO, 2021



original reports

Risk-Adapted Preemptive Tocilizumab to Prevent Severe Cytokine Release Syndrome After CTL019 for Pediatric B-Cell Acute Lymphoblastic Leukemia: A Prospective Clinical Trial

Stephan Kadauke, MD, PhD[1]; Regina M. Myers, MD[2,3]; Yimei Li, PhD[4]; Richard Aplenc, MD, PhD[2,3]; Diane Baniewicz, CRNP[2]; David M. Barrett, MD, PhD[2,3]; Allison Barz Leahy, MD[2,3]; Colleen Callahan, CRNP[2]; Joseph G. Dolan, MD[2,3]; Julie C. Fitzgerald, MD, PhD[5]; Whitney Gladney, PhD[6]; Simon F. Lacey, PhD[7]; Hongyan Liu, PhD[4]; Shannon L. Maude, MD, PhD[2,3]; Regina McGuire, BA[2]; Laura S. Motley, RN[2]; David T. Teachey, MD[2,3]; Gerald B. Wertheim, MD, PhD[1]; Lisa Wray, MD[2,3]; Amanda M. DiNofia, MD[2,3]; and Stephan A. Grupp, MD, PhD[1,2,3]

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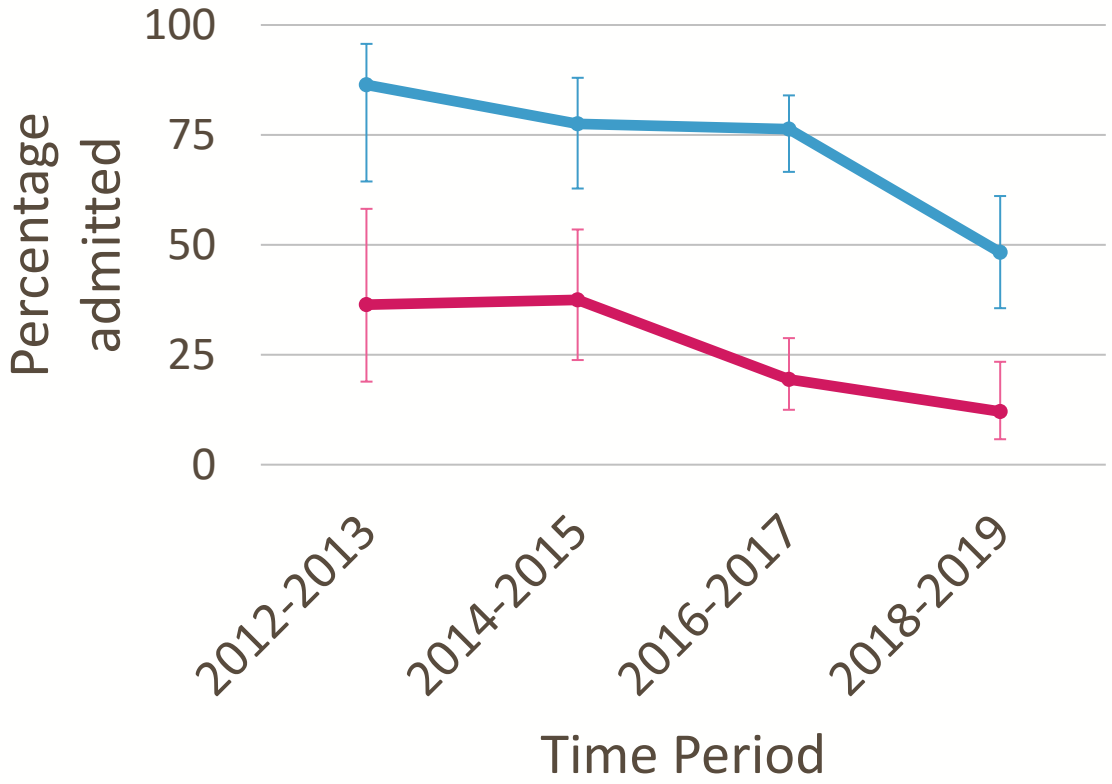
EARLY/PRE-EMPTIVE CRS TREATMENT

- BOR 97%:
  - high tumor burden cohort (>=40%) 87% CR/CRi
  - low tumor burden cohort (<40%) 100% CR/CRi
- Study endpoint: 2/3 reduction in grade 4 CRS
- Study endpoint met
- No impact on:
  - CR rate
  - CAR expansion
  - CAR persistence
  - ICANS (neurotoxicity)

Kadauke et al, JCO, 2021

TRENDS IN INPATIENT & ICU ADMISSION FROM 2012-2019



Inpatient Admission

$p < .001$

$p = 0.032$ (adjusted for HTB)

ICU Admission

$p = 0.001$

$p = 0.151$ (adjusted for HTB)

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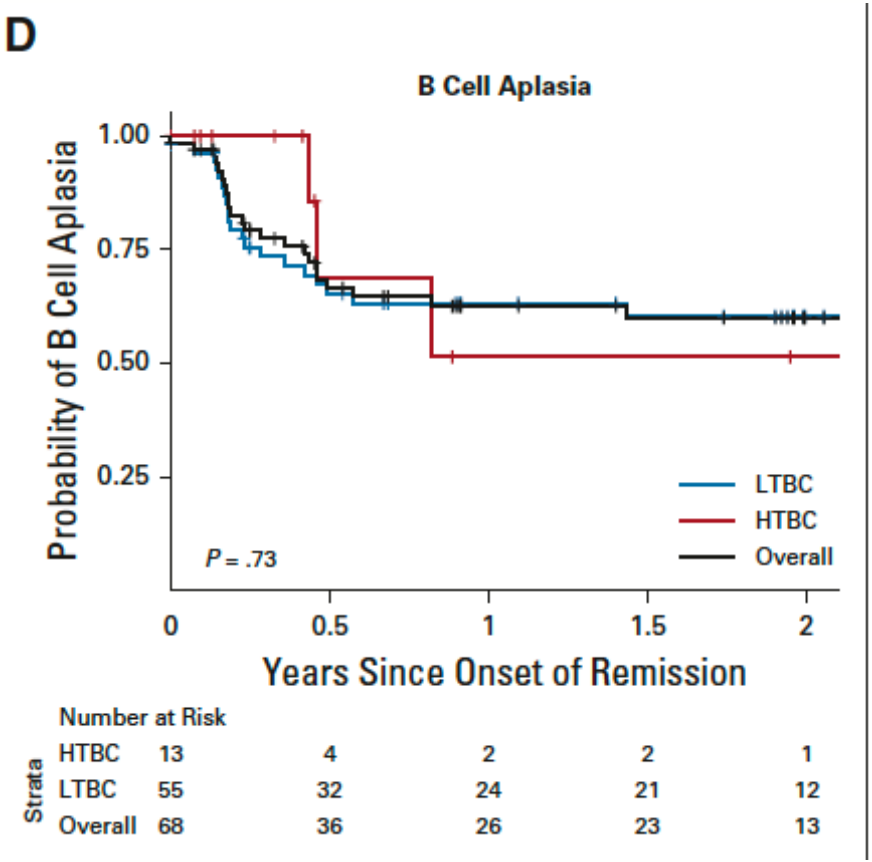
IS OUTPATIENT INFUSION OF CAR T POSSIBLE?

- >450 patients treated at CHOP
  (most ALL, most CD19 4-1BB CAR)
- >90% are infused in the outpatient clinic
- Observe for 1-2 hrs
- Each patient has a caretaker accompanying
- Stay within an hour (most are closer)
- 5 day/week CAR T clinic (not 7 days), see pts twice a week
- Admit for fever

PERSISTENT CAR T: BRIDGE TO SCT OR BRIDGE OVER SCT?

- 80% of CHOP patients do not go to BMT after CD19 CAR
- Monitor B cell aplasia monthly with peripheral CD19
- Bone marrow at 3 and 6 mo to look for MRD and hematogones
- Lower rate of CD19+ relapse as you get further out
- Reinfusion is an option, especially if B cell aplasia is lost after 3-4 months. Need LD chemo again.
- If the patient hits 6 mo and has B cell aplasia and is NGS MRD negative - very low relapse (Pulsipher, Blood Cancer Discovery)

PROLONGED B CELL APLASIA (4-1BB CD19 CAR)



Kadauke et al, JCO, 2021

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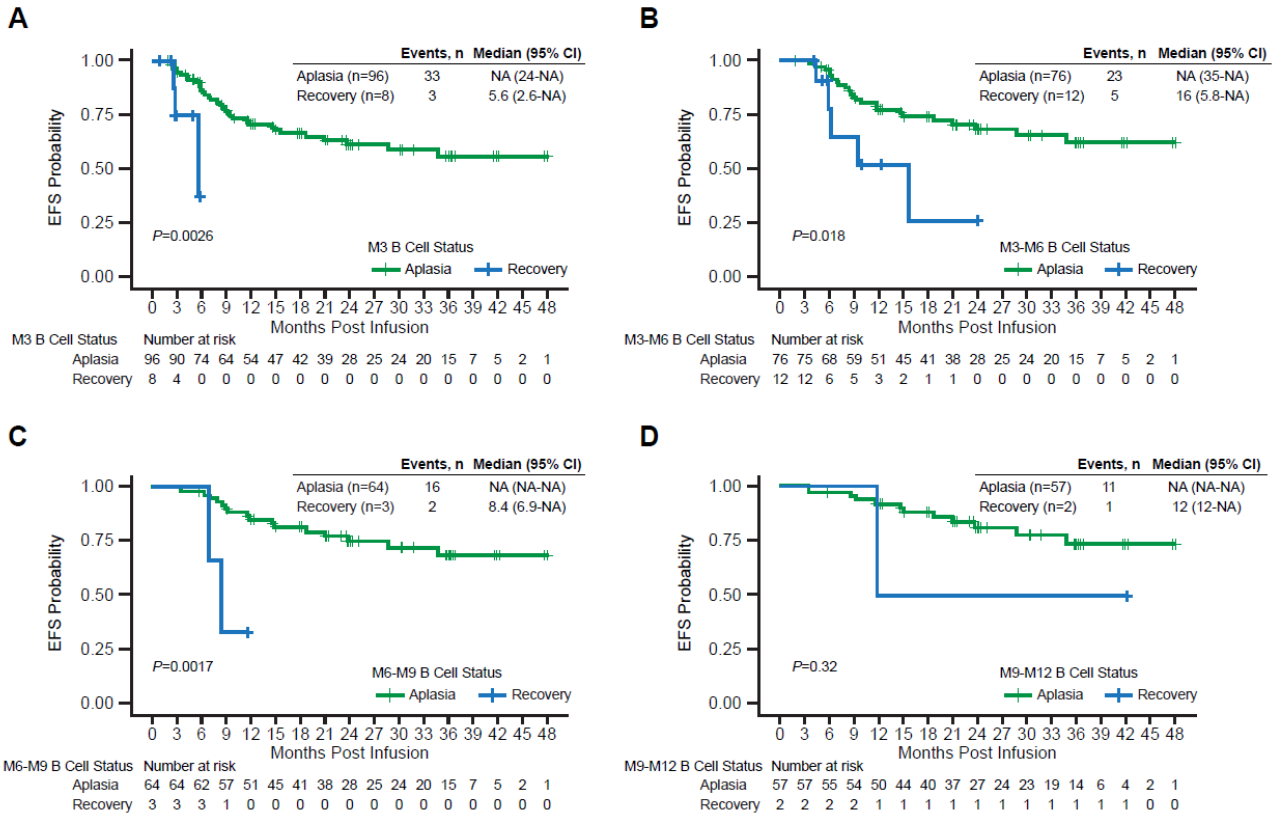
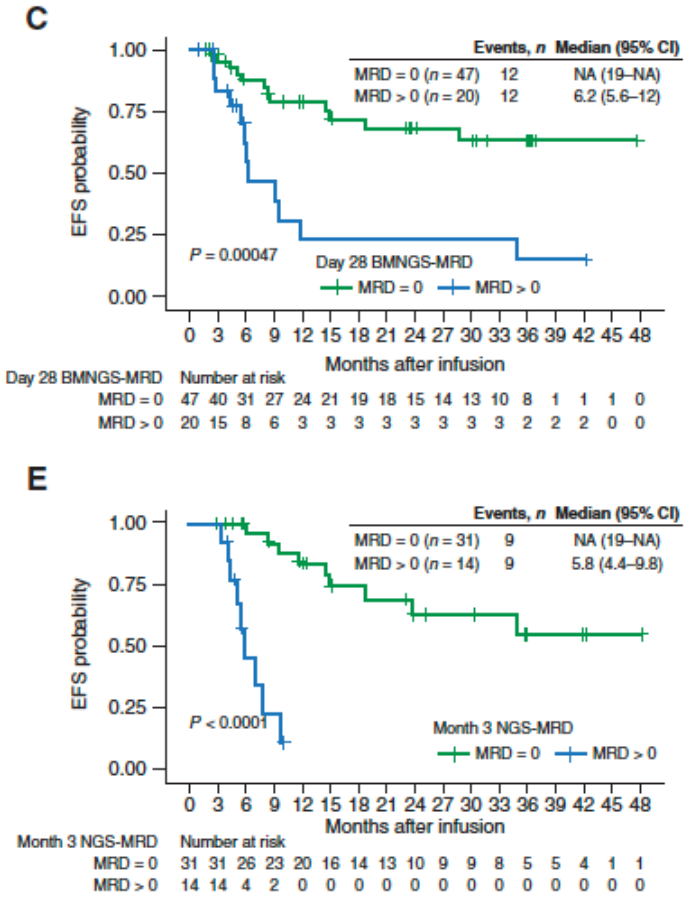
6 MONTHS IS AN INFLECTION POINT FOR RELAPSE RISK AFTER LOSS OF BCA (NOVARTIS ALL DATA)



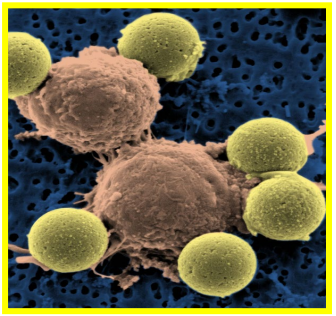
Pulsipher et al, Blood Cancer Discovery

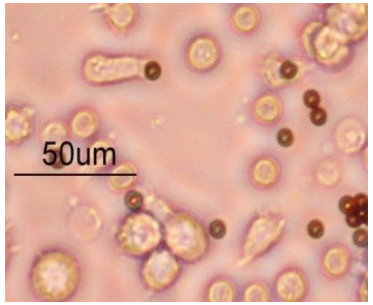
NGS MRD (ADAPTIVE CLONOSEQ) IS HIGHLY PREDICTIVE



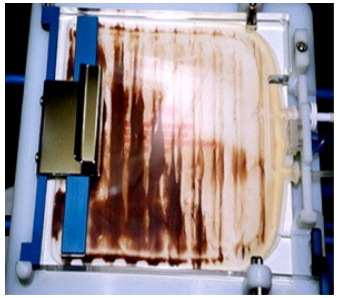
Pulsipher et al, Blood Cancer Discovery

Cell manufacturing matters





Bead addition



Bead removal

T-cell infusion

- **CD3/CD28 beads: clinical scale up, no feeder cells required**
- **Expansion >10^6-fold**
- **Repertoire preserved**
- **Maintains earlier T cell memory states**
- **Induction of telomerase: minimize replicative senescence**

Levine BL, et al. *J Immunol.* 1997; 159: 5921-5930.
Carroll RG, et al. *Science.* 1997; 276: 273-276.
Weng NP, et al. *Immunol. Rev.* 1997; 160: 43-54.
Humeau LM, et al. *Mol. Ther.* 2004; 9; 902-913.

GMP at UPenn CVPF –
Best in class academic CAR T manufacturing

GMP at CHOP – new closed manufacturing

Miltenyi Prodigy

Lonza Cocoon

- **Technology can help reduce FTE and space requirements**
- **RAPID MANUFACTURING**
- **Does short MFG time make better CAR T cells?**
- **Potential for ”point of care”?**

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ORAL ABSTRACTS

704.CELLULAR IMMUNOTHERAPIES: CLINICAL

A First-in-Human Study of YTB323, a Novel, Autologous CD19-Directed CAR-T Cell Therapy Manufactured Using the Novel T-Charge ™ platform, for the Treatment of Patients (Pts) with Relapsed/Refractory (r/r) Diffuse Large B-Cell Lymphoma (DLBCL)

Ian W. Flinn[1], Ulrich Jaeger[2], Nirav N. Shah[3], Didier Blaise[4], Javier Briones[5], Leyla Shune[6], Nicolas Boissel[7], Attilio Bondanza[8], Darlene Lu[9], Xu Zhu[9], Boris Engels[9], Jennifer L Brogdon[9], Jennifer Mataraza[9], Jaclyn Davis[10], Anne Laure Marchal[8], Luisa Mariconti[8], Michele Moschetta[8], Laure Moutouh-de Parseval[8], Pere Barba[11], Michael Dickinson[12]

- Based on CTL019/tisa-cel
- ~2 day MFG
- ? Length of release testing?
- Closed system
- May not need bridging therapy
- More Tscm and Tcm cells
- Much lower dose
- MFG starts in the GMP and finishes in the patient

- The ultimate ex vivo biomarker is a **POTENCY ASSAY**
- Currently no potency assay that predicts key aspects of in vivo performance:
  - Proliferation
  - Persistence

CD19 CAR Outcomes in ALL With CNS Involvement

CNS cohort

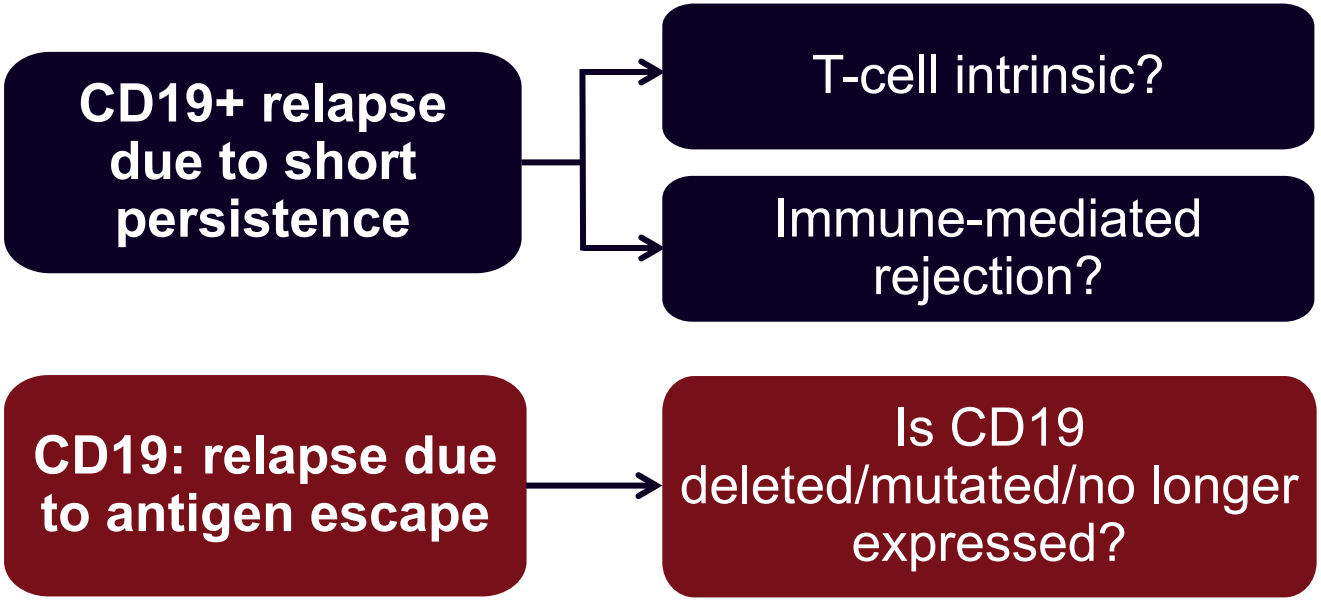
- 66 (34%) patients CNS3 within 12 months of infusion
  - Ranged from 1st to 6th relapse pre-CAR T
- 14 patients CNS2 and 4 patients CNS3 on day -1 (after LD)
  - 17 achieved CNS remission by 3 mo, 1 not evaluable
- 7/17 remain in continuous CR, 1 with SCT as long as 5 years
- Isolated CNS disease had better OS (91 vs. 71% at 2 years)

Entire cohort

- 98% of all patients treated have CTL019 detectable in CSF
- LPs out to 1 year demonstrate CTL019 in CSF
- 4% CNS relapses overall

1. Leahy, AB et al. *Lancet Hematology 2021*

Mechanisms of Relapse



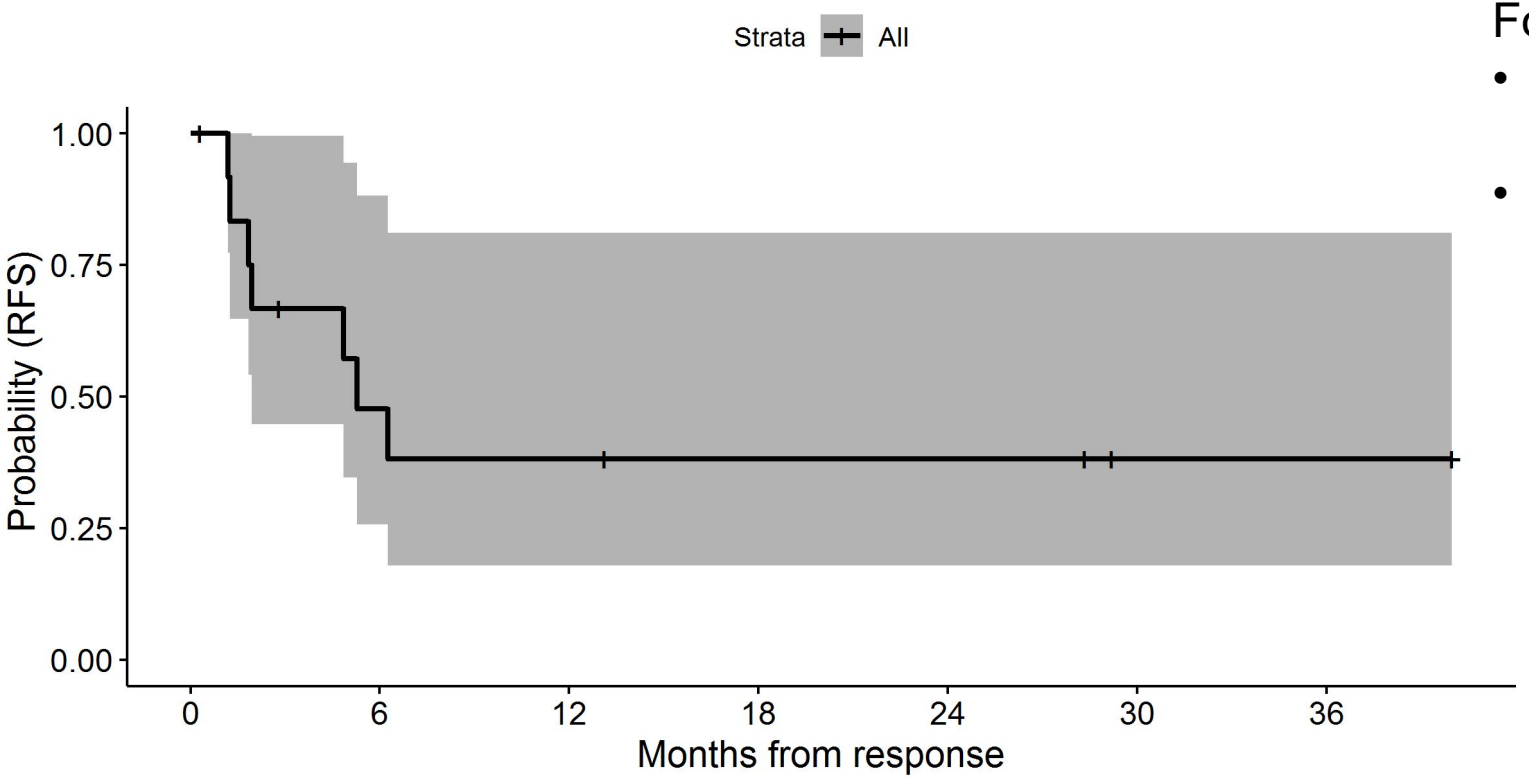
Exceptional Responder Project

- Patient Population:
  - Pediatric long-term survivors of CD19 CAR T cell therapy who 5-10 years from CAR T therapy and with no further therapy
  - We have >40 patients that received CD19 CAR 5-10 years ago with viably frozen apheresis, manufactured cells, multiple time points post-infusion
  - Comparator population: Patients who experienced CD19+ relapse within six months of CTL019 infusion

- CAR T cells to be sorted from longitudinal samples from each patient
  - Pre-infusion manufactured CAR T cell product
  - CAR T cells from days 7-14, 28 days post-infusion
  - CAR T cells from one-time large volume draw at >5 years from infusion

- Planned analysis:
  - Simultaneous scRNA-seq and scATAC-seq using the 10x Multiome pipeline

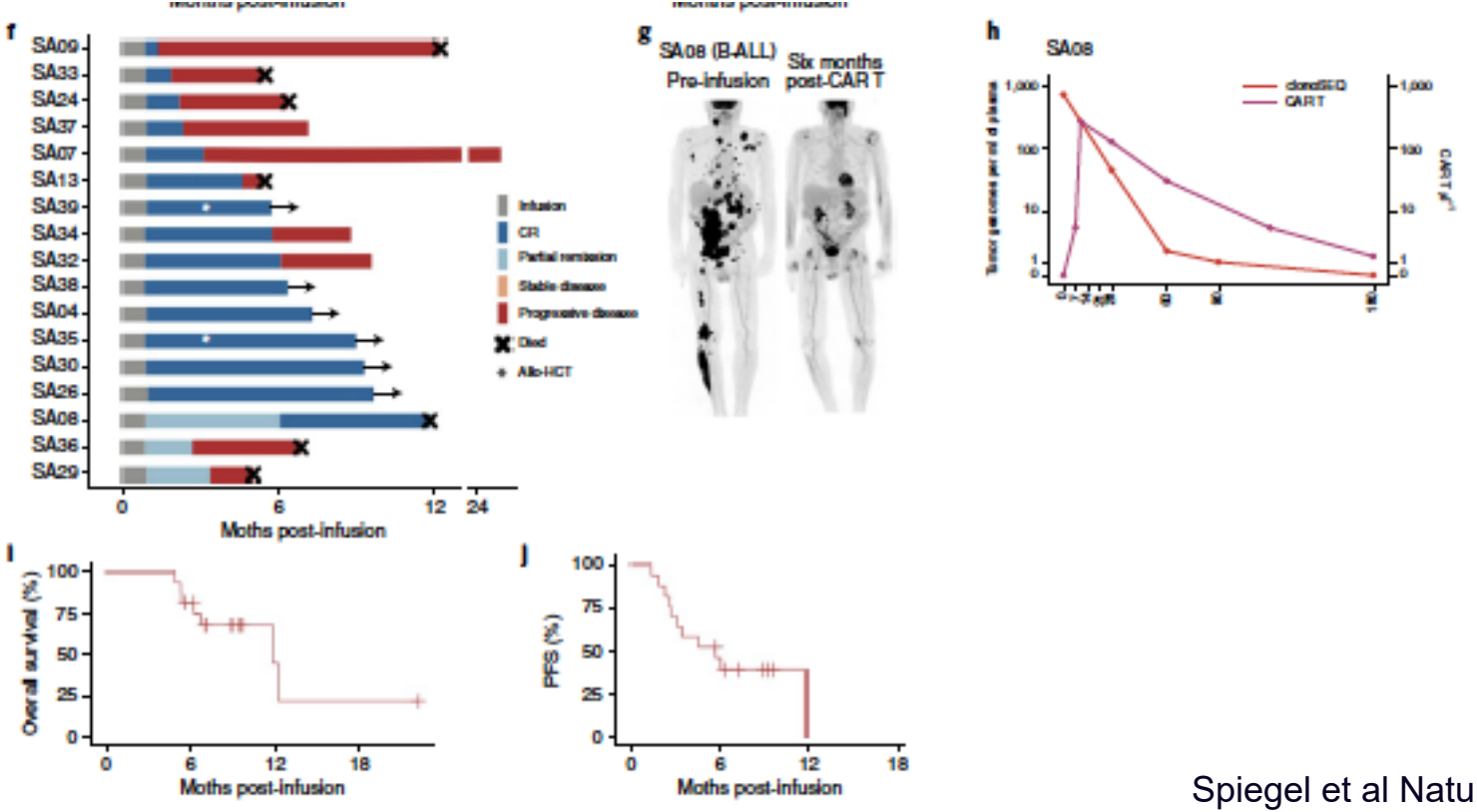
CART22-65s in CD19 escape ALL
CR rate 77%, RFS 40%



Follow up studies:

- CD19 CAR T + CD22 CAR T
- Tandem CD19/CD22 (Novartis T Charge MFG)

#1 problem in ALL CAR T is CD19 escape: Stanford bispecific CD19/CD22 CAR trial – ALL results



Spiegel et al Nature Medicine 2021

Penn Medicine

American Society of Hematology: 2021 Annual Meeting

CART22-65s Co-Administered with huCART19 in Adult Patients with Relapsed or Refractory Acute Lymphocytic Leukemia

Noelle V Frey, MD, MS[1], Saar Gill, MD, PhD[1], Wei-Ting Hwang, PhD[2*], Selina M. Luger, MD, FRCPC[1], Mary Ellen Martin, MD[1], Shannon R. McCurdy, MD[1], Alison W. Loren, MD[1], Keith W. Pratz, MD[1], Alexander E. Perl, MD[1], Julie Barber-Rotenberg, PhD[3*], Amy Marshall[3*], Marco Ruella, MD[1], Simon F Lacey, PhD[3], Joseph Fraietta, PhD[3*], Andrew Fesnak, MD[3*], Megan O'Brien[3*], Theresa Schanne[3*], Jennifer L Brogdon, PhD[4*], Boris Engels, PhD[4*], Bruce L Levine, PhD[3], Carl H June, MD[3], David L Porter, MD[1] and Elizabeth O. Hexner, MD[1]

[1]Cellular Therapy and Transplantation, Abramson Cancer Center of The University of Pennsylvania, Perlman School of Medicine

[2]Department of Biostatistics and Epidemiology, University of Pennsylvania

[3]Center for Cellular Immunotherapies, University of Pennsylvania, Philadelphia, The University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

[4]Novartis Institutes for BioMedical Research, Cambridge MA

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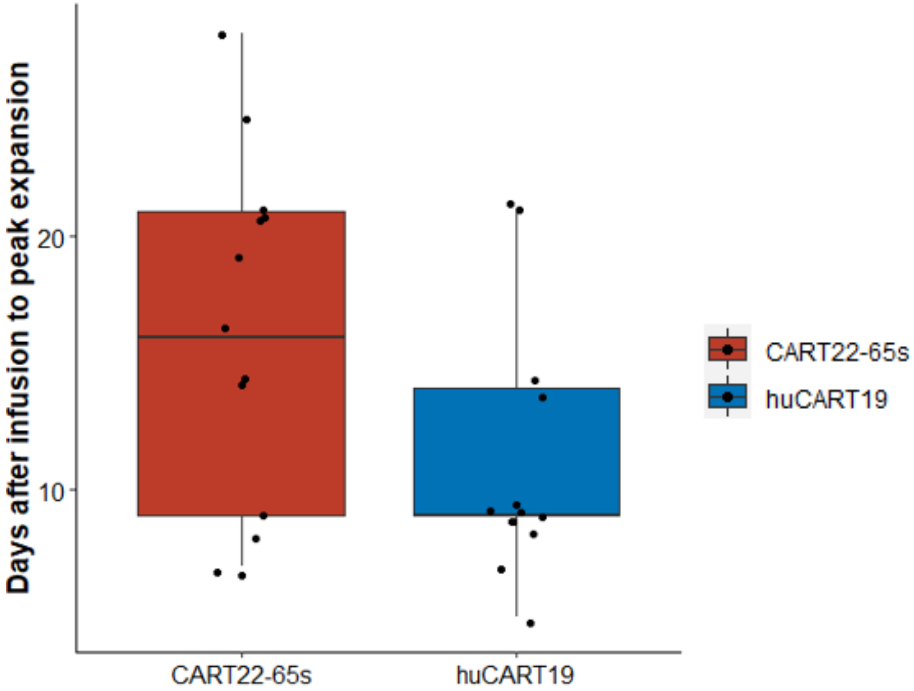
huCART19 and CART22-65s have distinct cellular kinetics

	Median (range) Days to Peak Expansion
CART22-65s	**16 (7-28)**
huCART19	**9 (5-21)**



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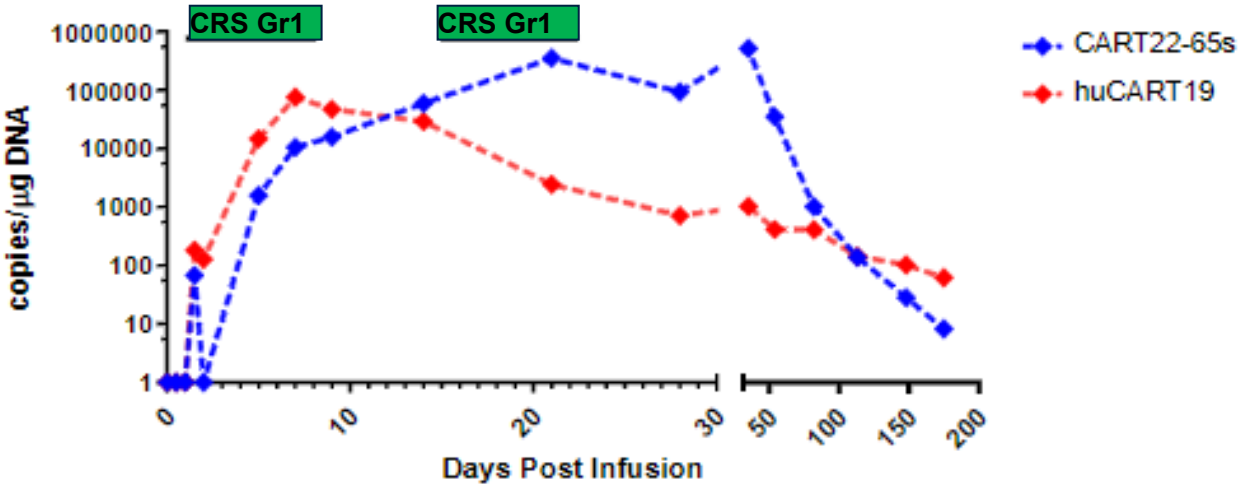
Different peak expansions correlate with distinct CRS events



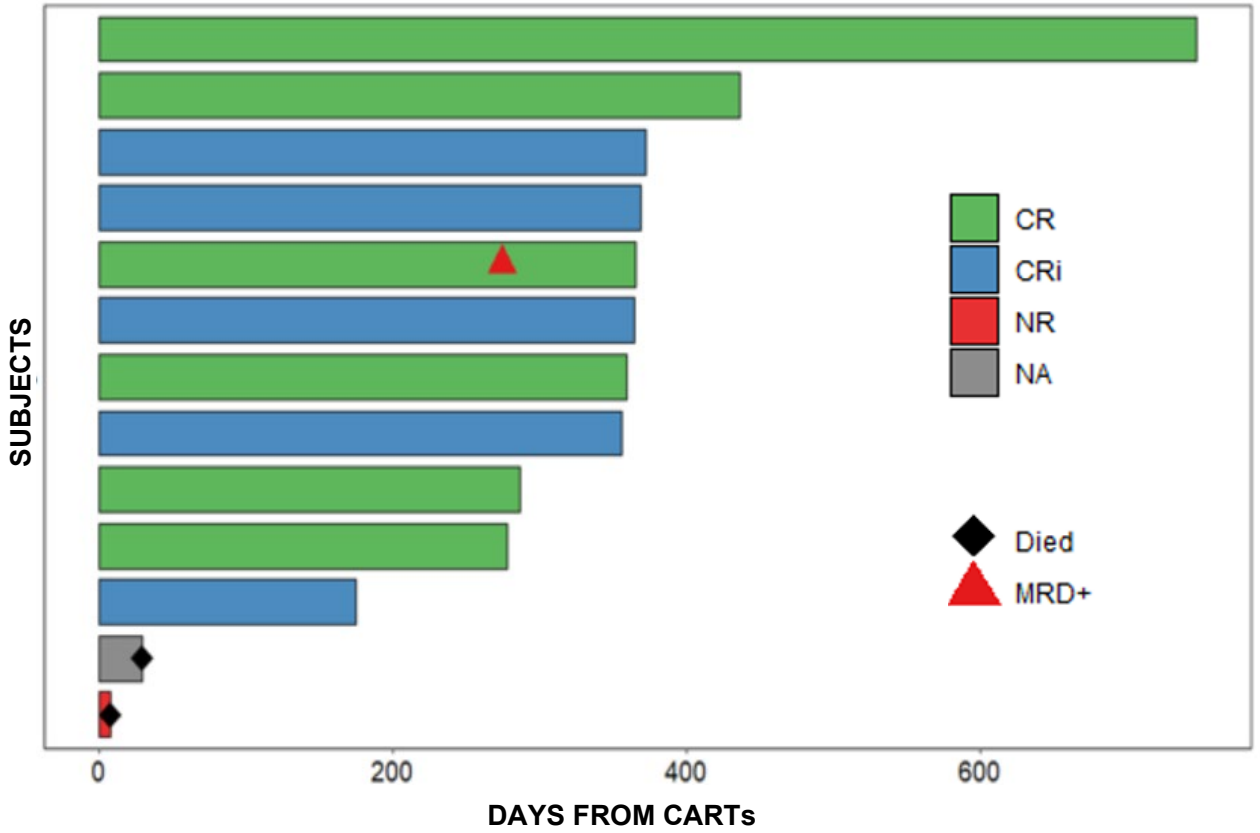
Response

CART19 and CART22:
(N=13)

- 13 pts infused
- 11 pts evaluable D28
- 11 CR/CRi (MRD -)

Med follow up 11.8 mo:

- One pt with molecular recurrence
- 10 with ongoing CR/CRi



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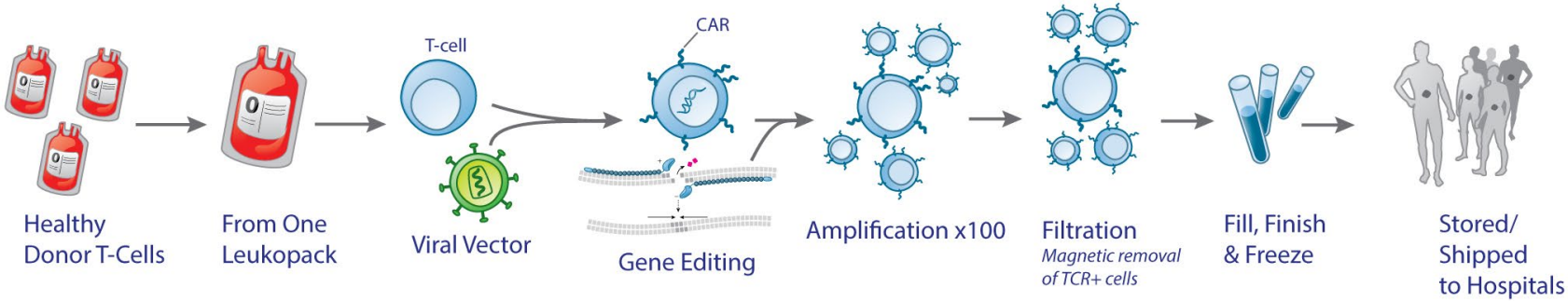
ALLOGENEIC CAR T-CELLS

The concept of off-the-shelf CAR T-cells



- Allogeneic CAR T-cells have the potential to benefit multiple patients from a single manufacturing batch
- Successful GMP manufacturing of UCART products
- Full QC system in place, cleared for clinical trials in the EU and by the FDA

One Leukopack can yield **100s of doses**

OFF THE SHELF CAR T: PROS

- Manufacturing cost could be much less
- Off the shelf = More rapidly available
- No need for patient apheresis, patient specific product
- No concerns about patient T cell status or prior chemo
- Multiple cell modifications much more feasible
- SO much easier, could be cheaper

- Neutral – probably similar CRS and neurotoxicity risks as autologous CARs

OFF THE SHELF CAR T: CONS

- Cells are allogeneic and subject to rapid rejection
- Is the fix reinfusion/multiple doses?

- GVHD risk – depends on TCR+ cells
- GVHD can include TaGVHD (transfusion-associated)
- May need stronger LD chemo +/- Campath
- True rejection will likely not be reversible; does rejection preclude reinfusion?

- Chromosomal aberrations are seen

Execution matters

Phari @Phari · Jul 7

Great **Abecma squeeze** continues.. about 1 in 9 pts get a slot to even collect T cells .. Its a travesty when an effective drug is just out of reach for waiting MM pts. My heart goes out to those who waited for CART approval @MediHumdani @BldCancerDoc @myelomacrowd @End_myeloma

NON-CANCER APPLICATIONS OF ENGINEERED CELL THERAPY

- FDA approved: Zynteglo for transfusion-dependent thalassemia, Skysona for cALD
- 2023: Lentiglobin and exa-cel for sickle cell disease
- These are autologous products which are gene modified bone marrow stem cells
- Examples of
  - gene insertion (beti-cel, Bluebird Bio) and
  - gene editing (exa-cel, Vertex)

Active Stem Cell Based Gene Therapy Clinical Trials for Non-Malignant Diseases

Hemoglobinopathie

Product	Sponsor	Strategy	Diseases	Age (y)
Lentiglobin BB305	bluebird bio	LV Gene addition: β-globin	SCD, BTM	2-50
BCH_BB-LCR shRNA(miR)	Boston Children's	LV Gene addition: shRNA targeting BCL11a	SCD	3-40
Lenti/G-βAS3-FB	UCLA	LV Gene addition: β-globin	SCD	>18
GLOBE1	Multiple	LV Gene addition: β-globin	SCD, BTM	5-35
TNS9.3.55	Mem Sloan Kettering	LV Gene addition: β-globin	BTM	>18
CSL200	CSL Behring	LV Gene addition: γ-globin + shRNA734	SCD	18-45
CTX001	Vertex	CRISPR-CAS9 Gene editing: BCL11a	SCD, BTM	12-35
OTQ923/HIX763	Novartis	CRISPR-CAS9 Gene editing: BCL11a	SCD	2-40
BIVV003	Bioverativ	ZFN Gene editing: BCL11a	SCD	18-40

Primary Immune Deficiencies

Product	Sponsor	Strategy	Diseases	Age (y)
OTL-101	Orchard	LV Gene addition: ADA	ADA-SCID	< 18
AProArt	UCSF	LV Gene addition: DCLRE1C	Artemis-SCID	> 2mth
SIN-LV-RAG1	Leiden Univ	LV Gene addition: RAG1	RAG-1 SCID	< 2
G2SCID	Boston Child	LV Gene addition: IL2RG	X-linked SCID	≤ 5
CL20-i4-EF1α-hγc-OPT	Multiple (Mustang)	LV Gene addition: IL2RG	X-linked SCID	varies
OTL-103	Orchard	LV Gene addition: WAS	Wiskott-Aldrich	> 5
G1XCGD	Genethon	LV Gene addition: CYBB	X-linked CGD	> 2

Neurologic, Metabolic, and BMF Disorders

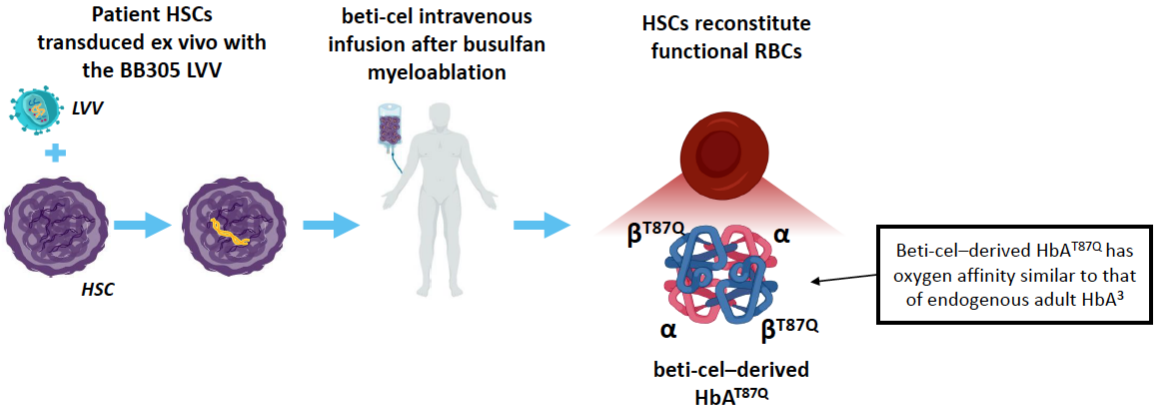
Product	Sponsor	Strategy	Diseases	Age (y)
OTL-200	Orchard	LV Gene addition: Arylsulfatase A	MLD	0-7
Lenti-D	bluebird bio	LV Gene addition: ABCD1	cALD	0-17
IDUA	IRCCS San Raffaele	LV Gene addition: α-L-iduronidase	MPS-1 (Hurler's)	0-11
RP-L401	Rocket	LV Gene addition: TCIRG1	Infantile Osteopetrosis	> 1mth
RP-L102	Rocket	LV Gene addition: FANCA	Fanconi Anemia	> 1

bbb beta-thal study – gene addition

Thompson et al. ASH 2021. Abstract 148177

Betibeglogene autotemcel (beti-cel) ex vivo gene therapy for TDT

- Transfusion-dependent β-thalassemia (TDT) is a severe, progressive genetic disease caused by mutations in the β-globin gene resulting in absent or significantly reduced adult hemoglobin, HbA, that normally accounts for approximately 95% of the total Hb in the blood of adults after 6 months of age[1,2]



1. Paramore C, et al. *Patient.* 2021;14(2):197-208. 2. Thomas C, et al. *Continuing Education in Anaesthesia. Critical Care & Pain.* 2012;12(5):251-6. 3. Pawliuk R, et al. *Science.* 2001;294(5550):2368-71.
HbA, adult hemoglobin; Hb, hemoglobin; HSC, hematopoietic stem cell; LVV, lentiviral vector; RBC, red blood cell; TDT, transfusion-dependent β-thalassemia.

Lentiviral transduction concerns

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Acute Myeloid Leukemia Case after Gene Therapy for Sickle Cell Disease

Sunita Goyal, M.D., John Tisdale, M.D., Manfred Schmidt, Ph.D., Julie Kanter, M.D., Jennifer Jaroscak, M.D., Dustin Whitney, Ph.D., Hans Bitter, Ph.D., Philip D. Gregory, Ph.D., Geoffrey Parsons, Ph.D., Marianna Foos, M.S., Ashish Yeri, Ph.D., Maple Gioia, A.L.M., Sarah B. Voytek, Ph.D., Alex Miller, B.S., Jessie Lynch, M.S., Richard A. Colvin, M.D., Ph.D., and Melissa Bonner, Ph.D.

bluebird bio Announces Temporary Suspension on Phase 1/2 and Phase 3 Studies of LentiGlobin Gene Therapy for Sickle Cell Disease (bb1111) (news release). Cambridge, MA: bluebird bio, Inc; Feb. 16, 2021

CRISPR EDITING TRIAL FOR HEMOGLOBINOPATHIES

ASH 2020: CRISPR and Vertex's Potential Cure for Sickle Cell Disease and More Glimmers of Hope

Published: Dec 07, 2020 | By Mark Terry

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia

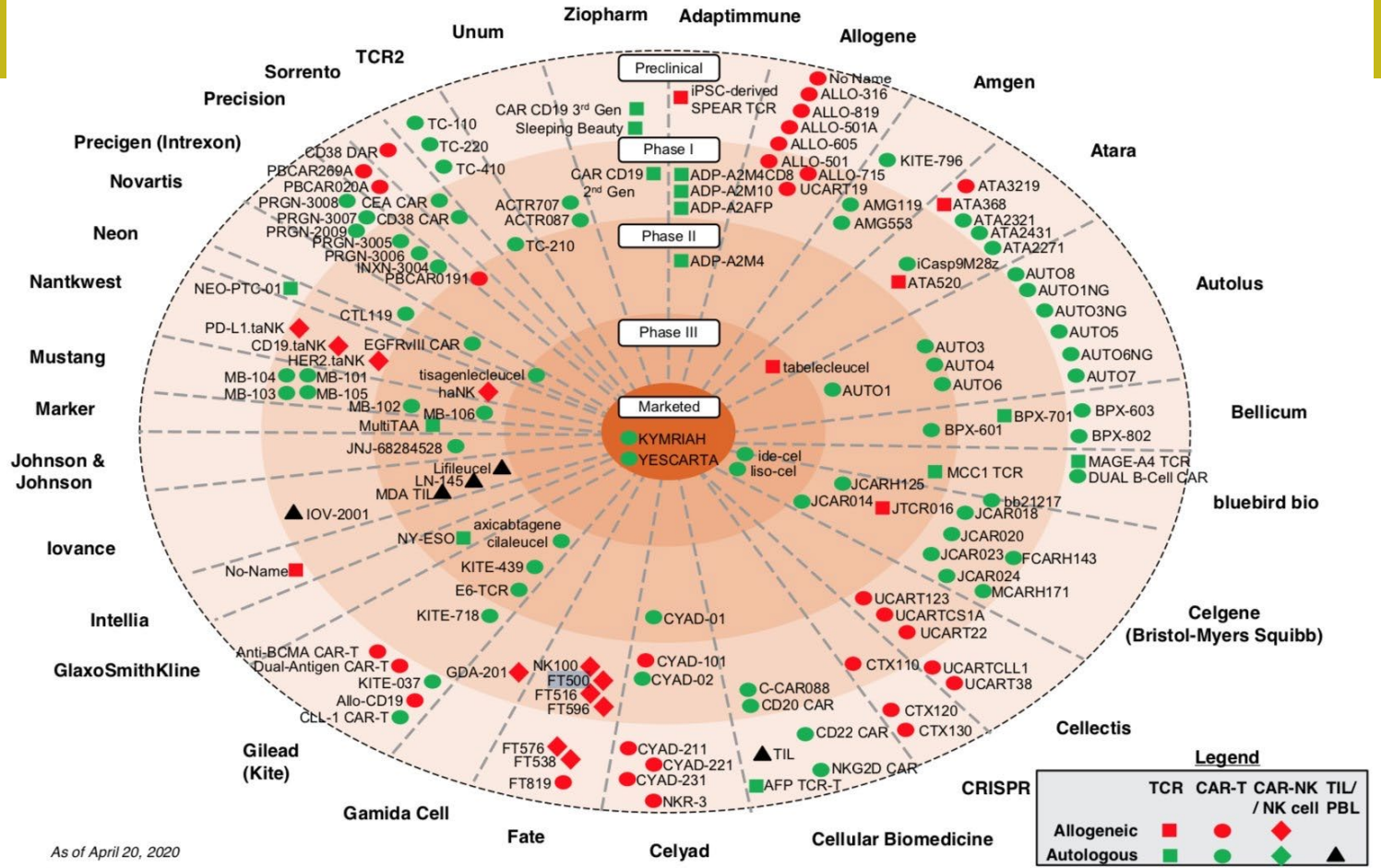
H. Frangoul, D. Altshuler, M.D. Cappellini, Y.-S. Chen, J. Domm, B.K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T.W. Ho, A. Kattamis, A. Kernytsky, J. Lekstrom-Himes, A.M. Li, F. Locatelli, M.Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M.H. Steinberg, D. Wall, A. Yen, and S. Corbacioglu

Children's Hospital of Philadelphia®
Cellular Therapy & Transplant

Opportunities for Improvement in Cell Therapy

- Cost!
- Manufacturing is first generation. There are HUGE opportunities for better MFG – speed, success rates, cost of goods, automation
- How do we pay for this?
  - Kymriah (tisa-cel) $475K (ALL), Zolgensma $2.1M
    Zynteglo (beti-cel) $2.8M (US)
  - Value-based pricing:
    tisa-cel (ALL) has a value/outcome based agreement
  - Pay over time models

Key Cellular Therapy Assets in Development - Competitive Landscape In Oncology



As of April 20, 2020