CAR T-cell therapy

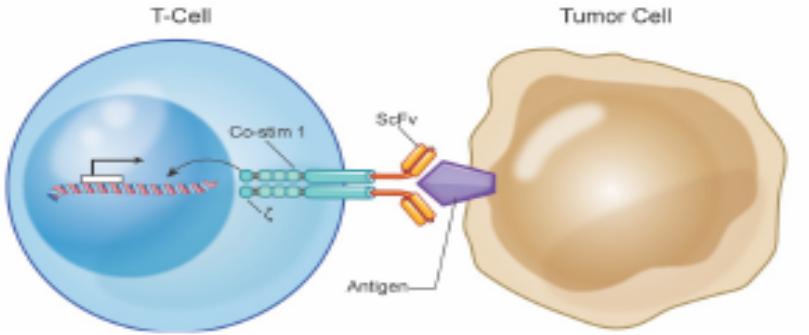
Objectives

Objectives

- Basic overview of the CAR T-cell program in children and young adults
- Current status of CAR T-cell therapy in pediatric ALL
- Review limitations and active efforts to address these challenges
- Discuss future directions

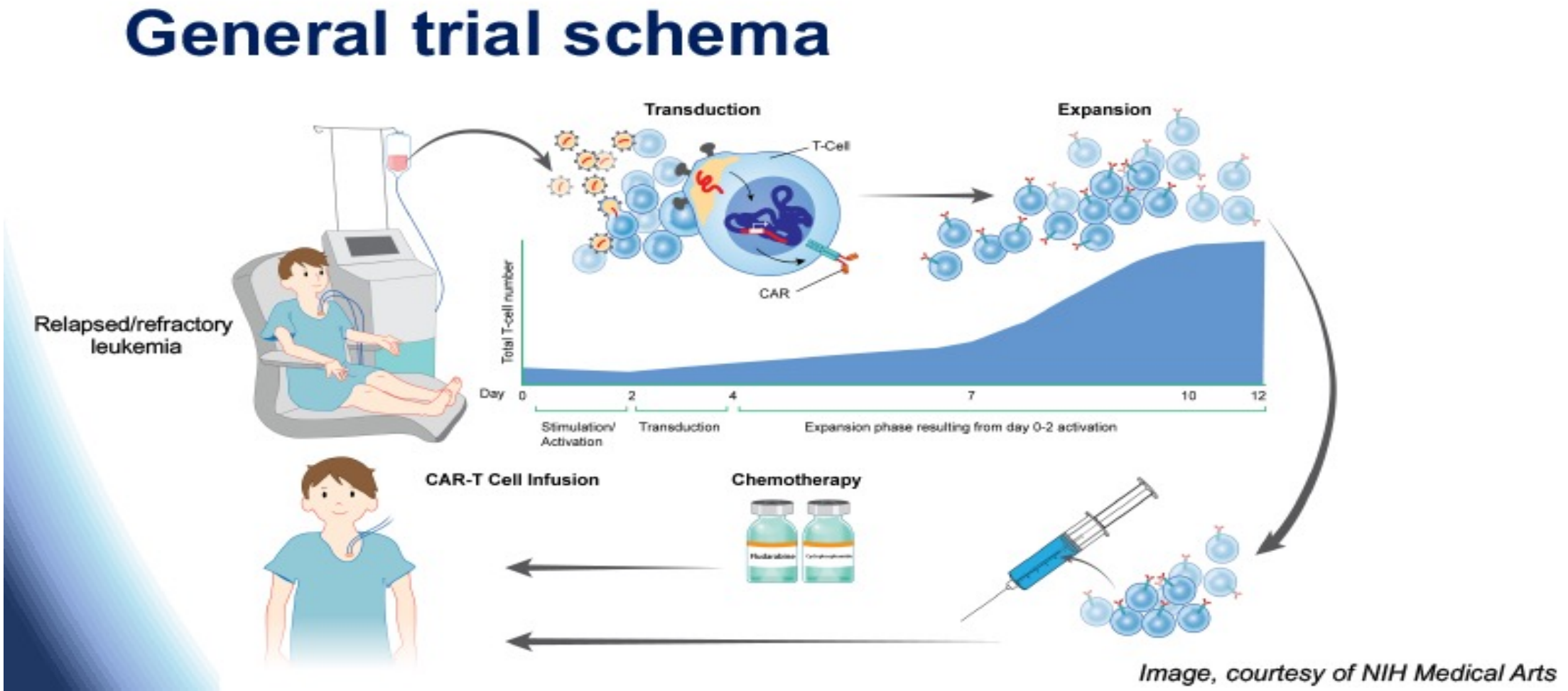
Adoptive cell therapy

CAR T-cell therapy

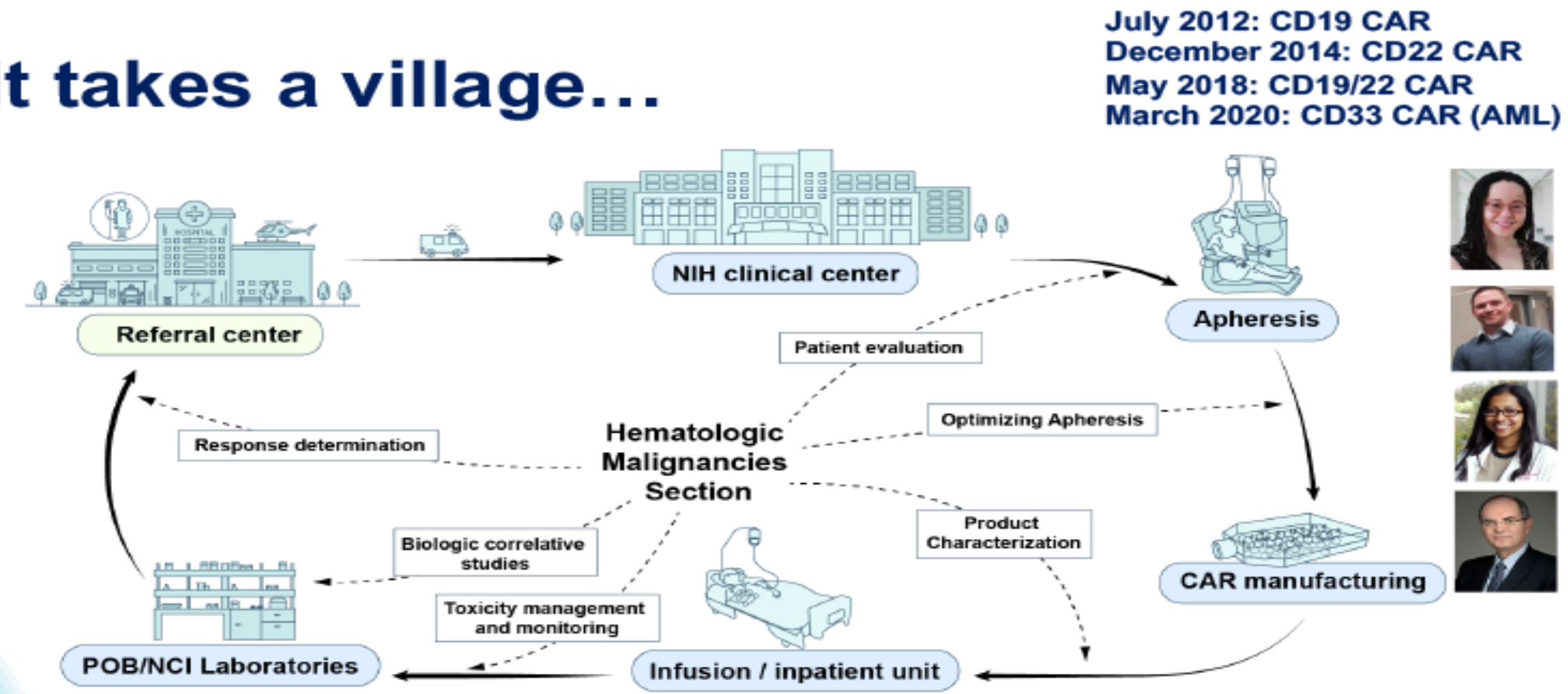


- Adoptive cell therapy
  - Mechanism to overcome the inherent inhibition of endogenous T cells to target and eliminate cancer cells
  - Engineered T cells provide enhanced specificity and efficacy to target cancer
- MHC independent recognition of cell surface antigens
  - CD19/CD22
- Built in co-stimulatory signaling domains
  - 4-1BB or CD28 with CD3z
- T-cell functionality coupled to antibody based antigen recognition

Schema


General trial schema
Transduction
Expansion
T-Cell
CAR
Relapsed/refractory leukemia
Total T-cell number
Day
0
2
4
7
10
12
Stimulation/ Activation
Transduction
Expansion phase resulting from day 0-2 activation
CAR-T Cell Infusion
Chemotherapy
Image, courtesy of NIH Medical Arts

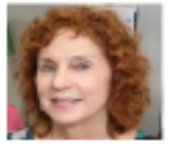
It takes a village



Novel immunothreapy

Extensive correlative studies are embedded in the infrastructure of these novel immunotherapies

- **Cytokine profiling**
- **CAR T-cell trafficking, persistence and expansion**
  - Blood, bone marrow and CSF
- **Toxicity and response monitoring**
  - Routine clinical laboratory evaluations
  - Anti-cytokine directed therapy
  - Prospective neurotoxicity evaluations
  - Patient reported outcomes
  - Adverse event monitoring
  - Imaging
- **Leukemia biology**
  - Evaluation of CD19/22 expression
  - Lineage switch
  - Immunophenotypic evaluations
  - Genomics
- **Optimization Strategies**
  - Manufacturing
  - CAR T-cell product analysis
  - Toxicity mitigation
  - Immunogenicity
- **Highly collaborative network**

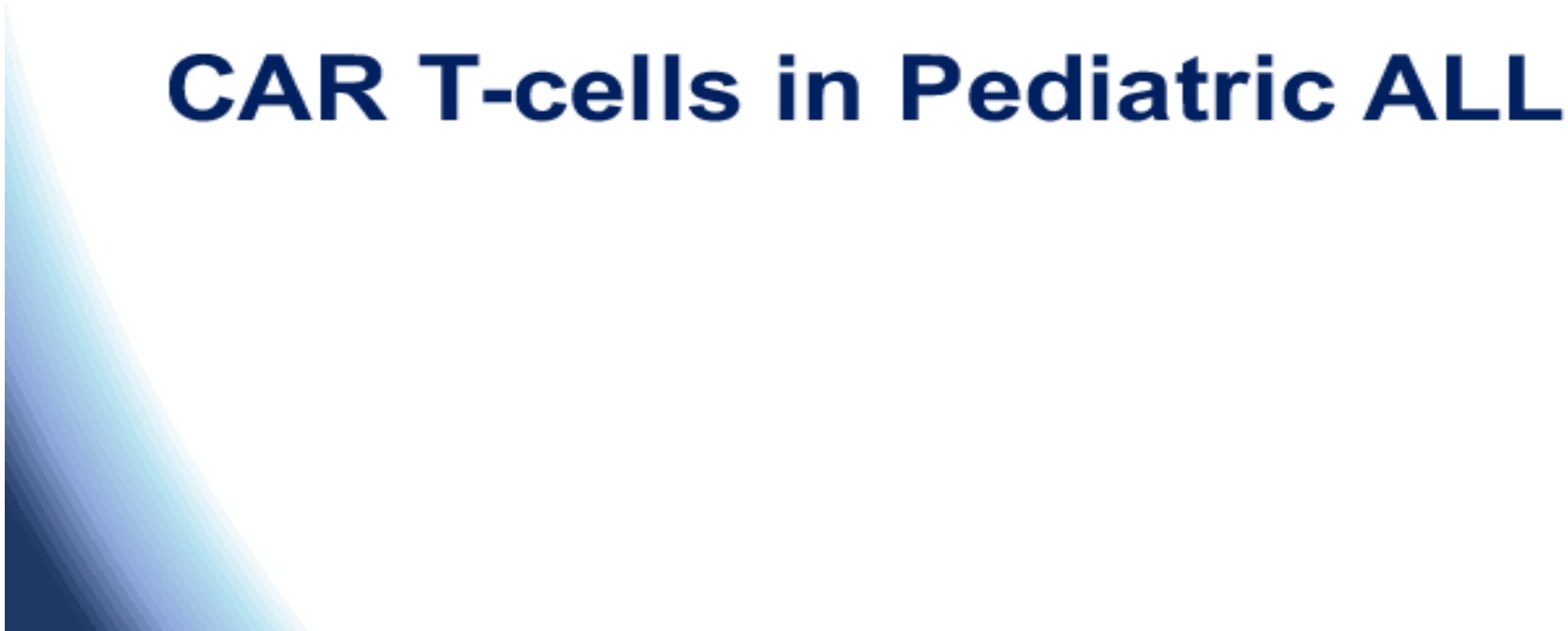






Collaboration with NCI Flow Cytometry, POB (Naomi Taylor, Pam Wolters, Staci Martin), Center for Cellular Engineering, FNLCR

Pediatric ALL



Pediatric ALL

Pediatric ALL: Outcomes for relapsed/refractory disease

- Acute lymphoblastic leukemia (ALL) is the most common childhood cancer
- "Poster-child" for success in cancer therapy due to cooperative group efforts
- 85-90% cure rates
- Those with relapsed/refractory disease have poor outcomes

Table 4 Comparison of unadjusted CR rates of patients with medullary relapsed/refractory ALL between two sequential TACL studies

Number of salvage attempt	CR rate (SE) [95% confidence interval]		Difference (Sun–Ko) (SE) (testing proportion)
	1995–2004 (Ko et al.) [5]	2005–2013 (Sun et al.)	
Second salvage attempt	44.44 % (4.78) [34.88, 54.32]	50.91 % (3.89) [43.02, 58.76]	0.0647 (0.0616) (−0.0561, 0.1855) $p = 0.2955$
Third salvage attempt	26.78 % (5.92) [15.83, 40.30]	36.99 % (5.65) [25.97, 49.09]	0.1021 (0.0818) (−0.0583, 0.2624) $p = 0.2200$
Fourth through eighth salvage attempt	12.31 % (4.07) [5.47, 22.82]	30.77 % (6.40) [18.72, 45.10]	0.1846 (0.0759) (0.0358, 0.3333) $p = 0.0140$

CR complete remission, *SE* standard error

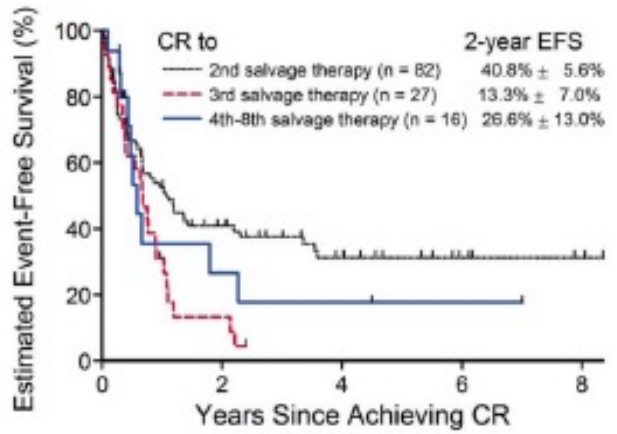


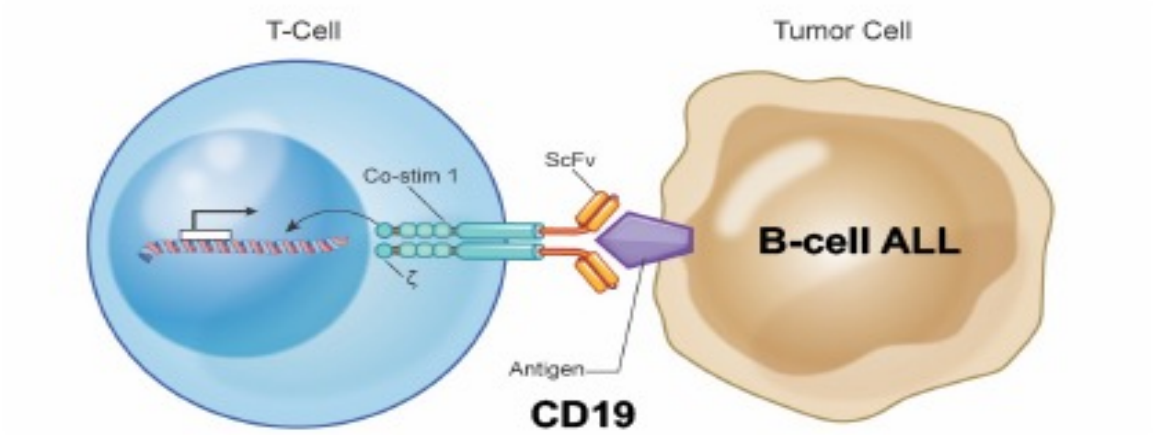
Fig. 2 Estimated 2 year event-free survival for patients who achieved complete remission after ≥2nd salvage attempt. CR complete remission, EFS event-free survival

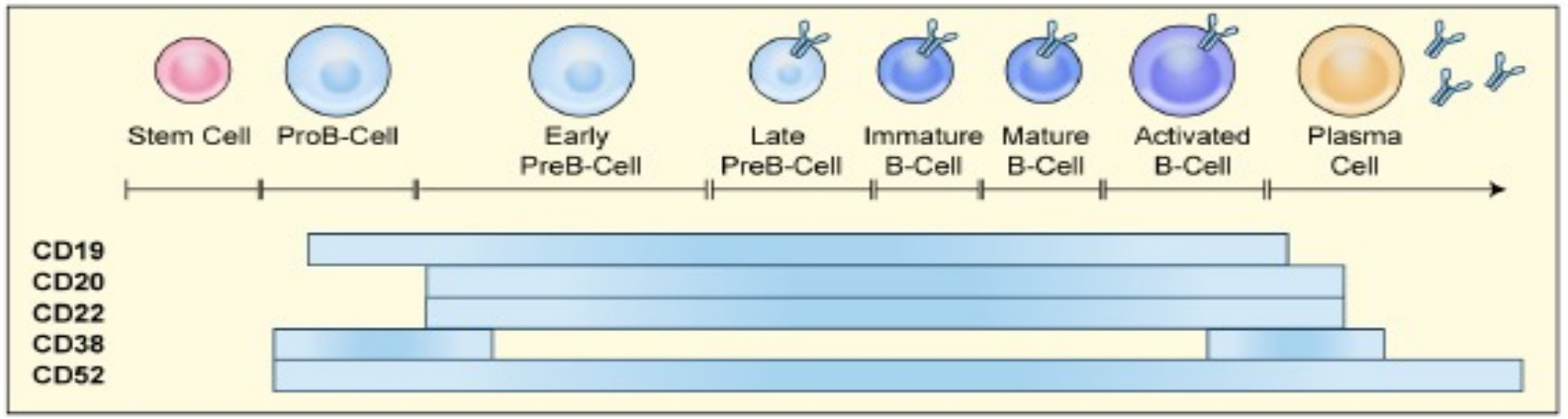
Sun/Whitlock, Leukemia, 2018

Targeting CD19

Targeting CD19

- CD19 ubiquitously found on B-cells

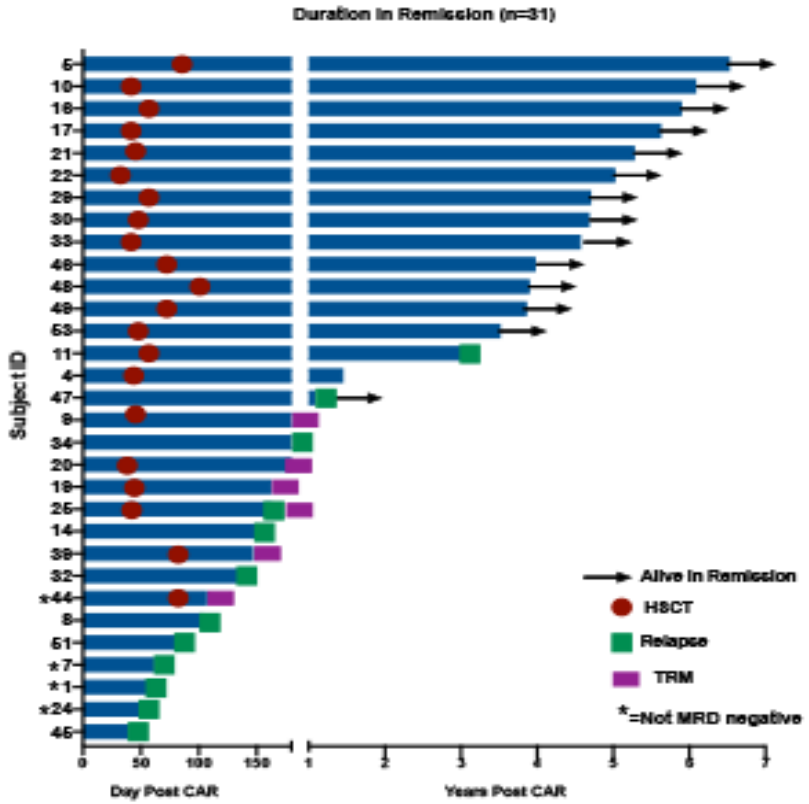




CD19 CAR

CD19 CAR (Pediatric Oncology Branch)

- July 2012
  - CD19-28ζ (now Yescarta)
- Lessons learned:
  - Cytokine release syndrome
  - CAR T-cell persistence
  - Importance of fludarabine/cyclophosphamide
  - Treatment of active CNS disease
  - Role of stem cell transplant
- Changed the paradigm for phase 1 trials re: response

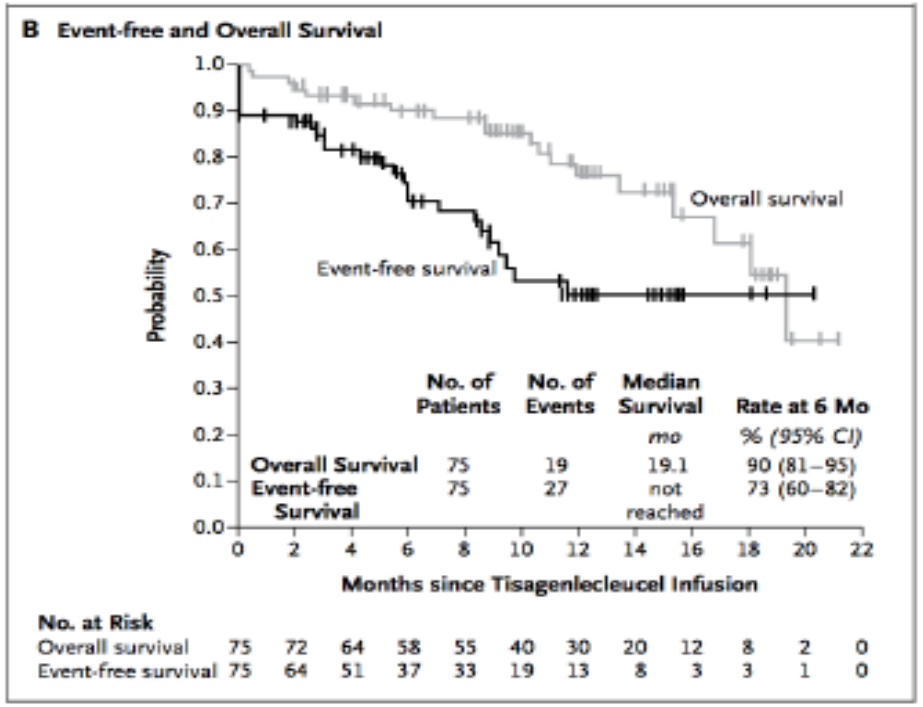


Shah NN et al., J Clin Oncol, In Press

Clinical updates

CD19 CAR clinical updates (Kymriah)

- 81% Complete remission rate
  - Children with relapsed/refractory B-cell ALL
  - CD19/4-1BB (Children's Hospital of Philadelphia)
  - Tisagenlecleucel
- Event Free Survival:
  - 6 months: 73%
  - 12 months: **50%**



Maude SL, et al. NEJM 2018

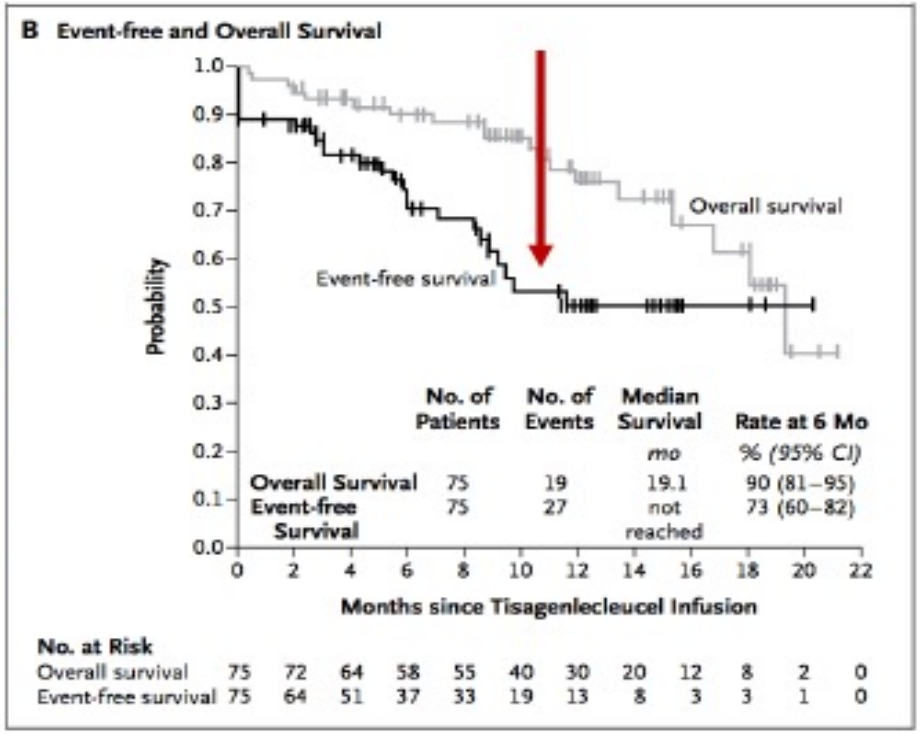
FDA approvals

FDA approvals: CD19 CAR T-cells

- **Kymriah® (tisagenlecleucel):**
  - **Pediatric B-ALL (up to age 25)**
  - Adults with Large B-Cell lymphoma
- Yescarta® (axicabtagene ciloleucel):
  - Adults with Large B-cell lymphoma
- Tecartus™ (brexucabtagene autoleucel):
  - Mantle Cell lymphoma

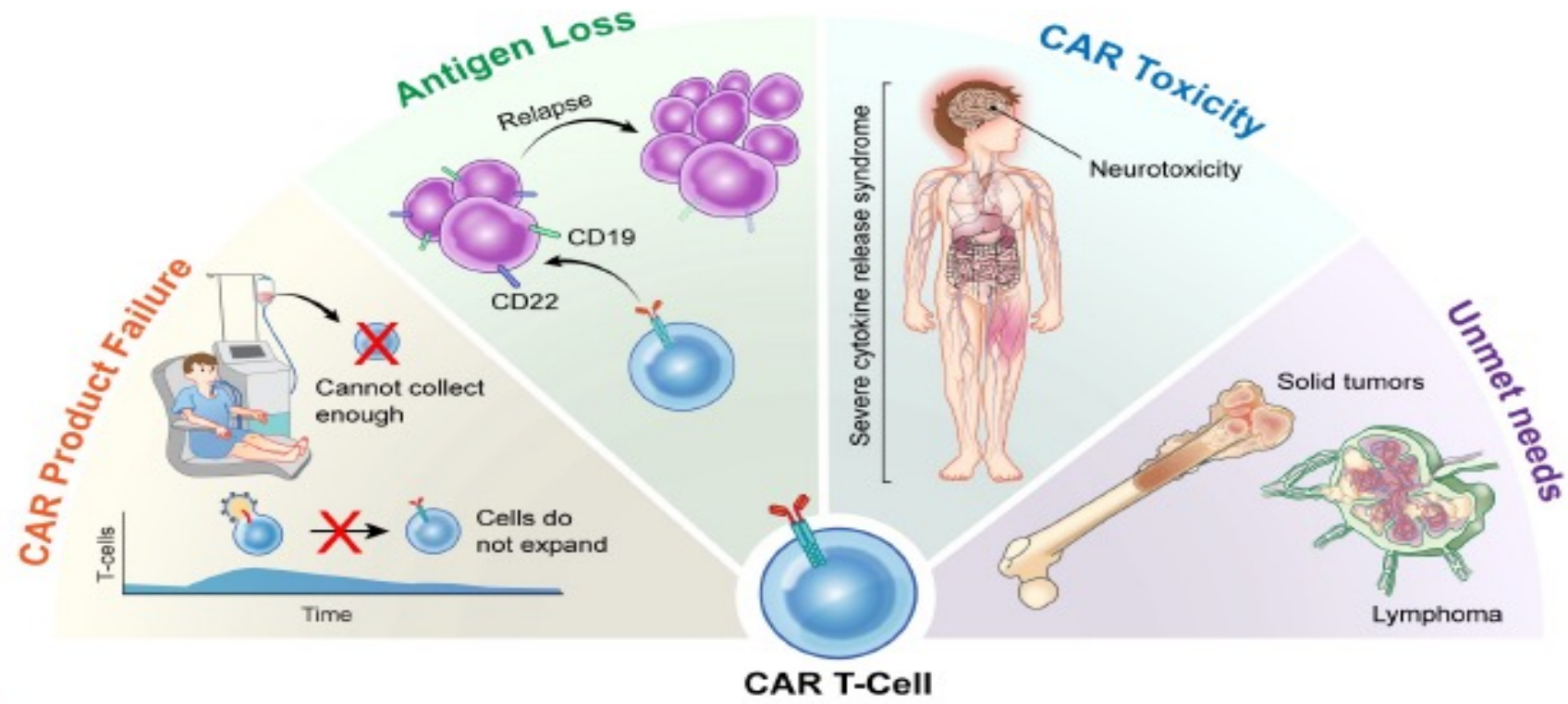
CURE

Will CD19 CAR T-cells be the CURE?



https://emilywhiteheadfoundation.org/

Current challenges



Shah NN, Nat Rev Clin Oncol

Current limitations

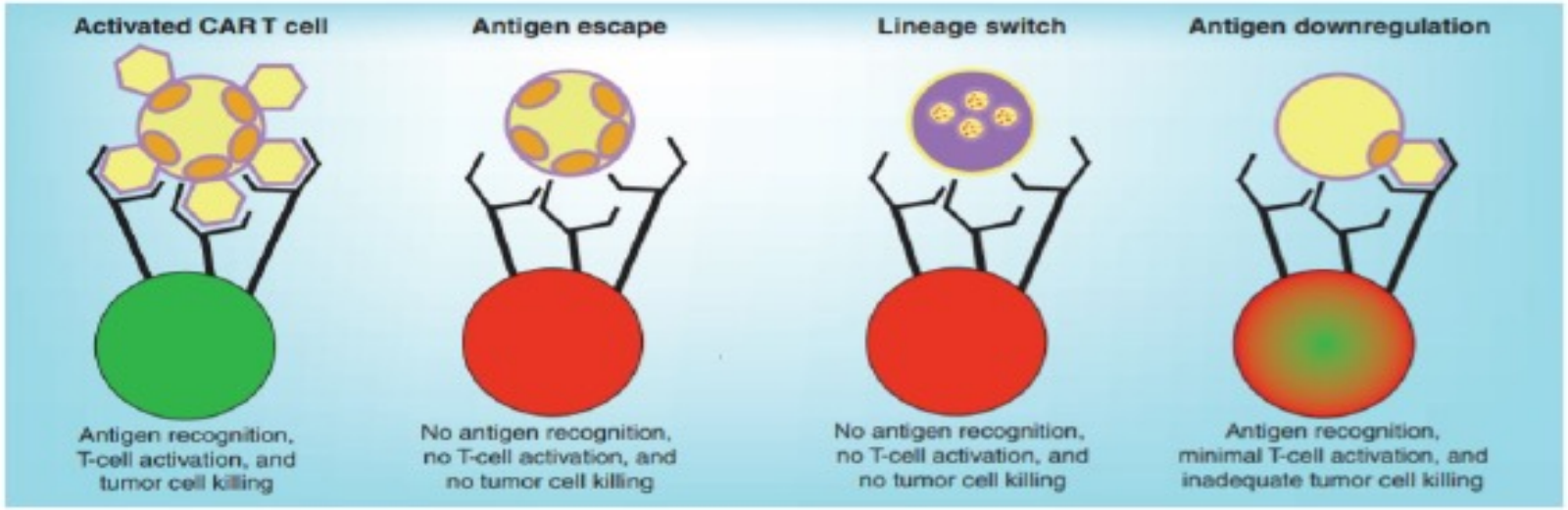
Current limitations

- Relapse: With or without the surface antigen (CD19)
  - Problem 1 (CD19+): Second CAR infusions generally don't work as well
  - Problem 2 (CD19 neg): If you don't see it, you can't treat it
- Manufacturing: If you can't make it, you can't use it
- Toxicity: Need to survive it
- Disease: Going beyond ALL

CD19 loss

Catch me if you can!!!

- CD19 loss or down regulation represents the primary form of treatment failure

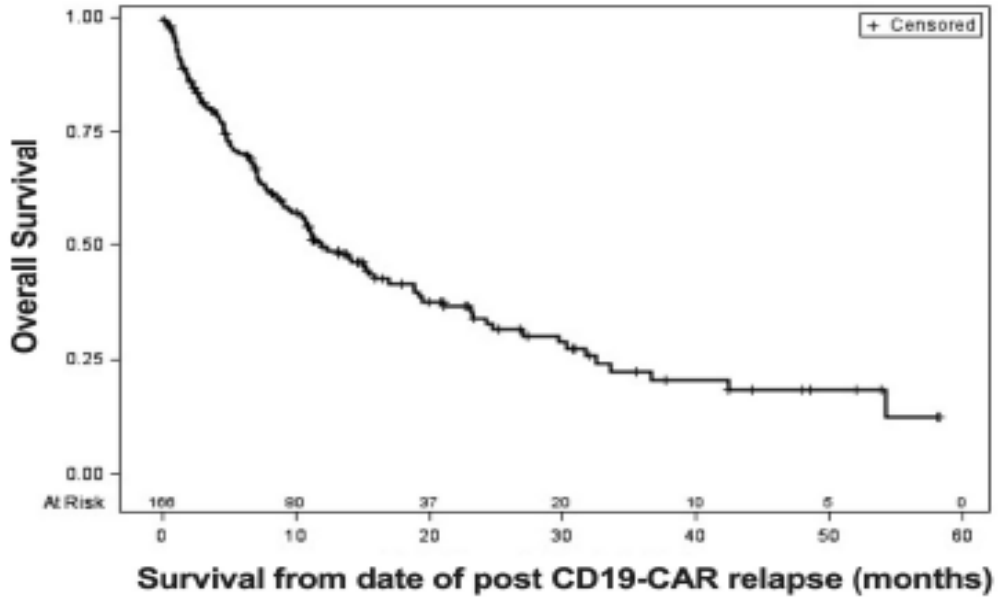


Majzner R., Cancer Discovery, 2018

Outcomes

Outcomes for post CD19 CAR relapse are poor

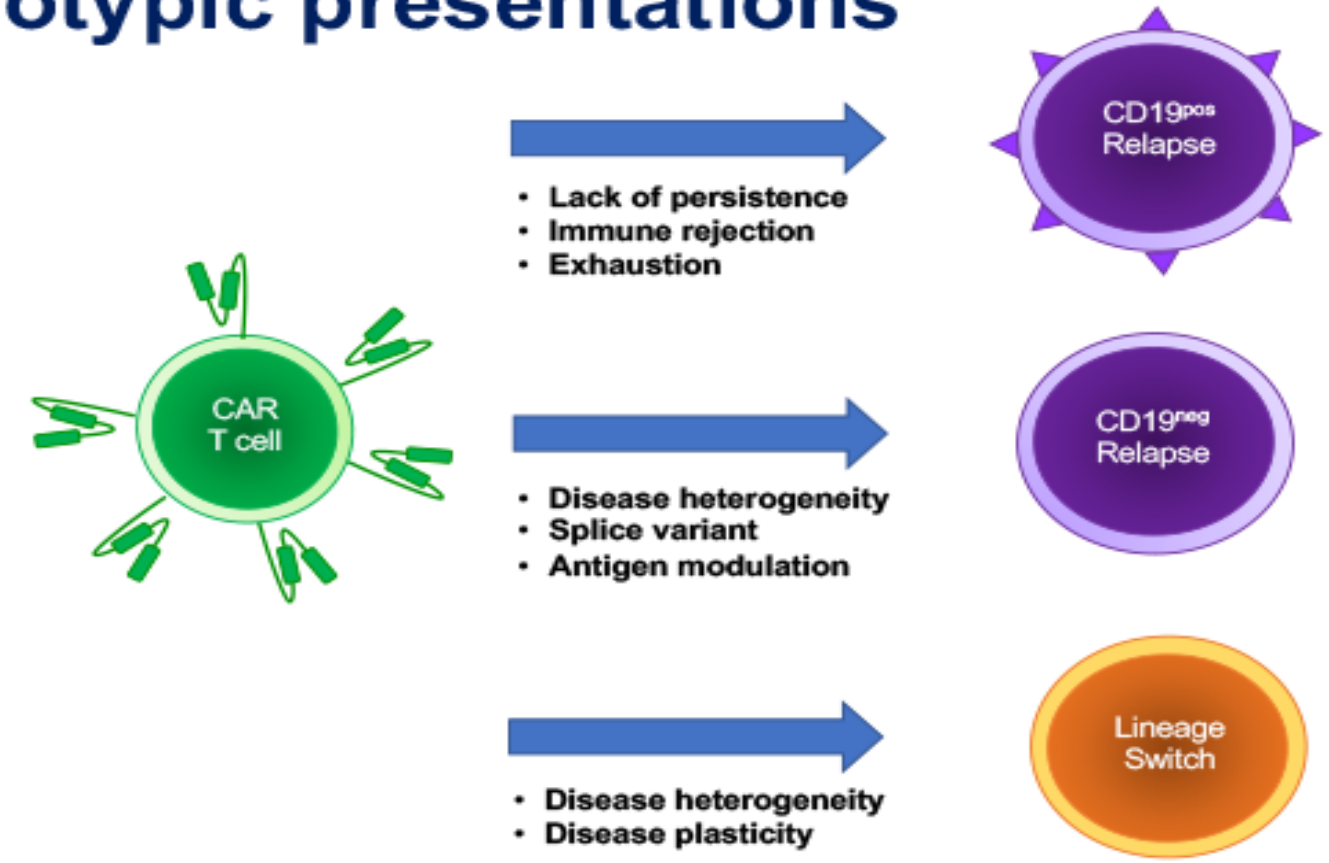
- Retrospective, multicenter study of 420 children and young adults receiving CD19 CAR T-cells
  - 166 (39.5%) with relapse
- Median overall survival (OS):
  - 11.9 months (95% CI: 9.0-17.9 mo)
- 12 month OS: 49.4%
- Salvage options, limited
  - Particularly for CD19^{neg} relapse



Lamble A/Shah NN, Blood Adv, 2022

Etiology

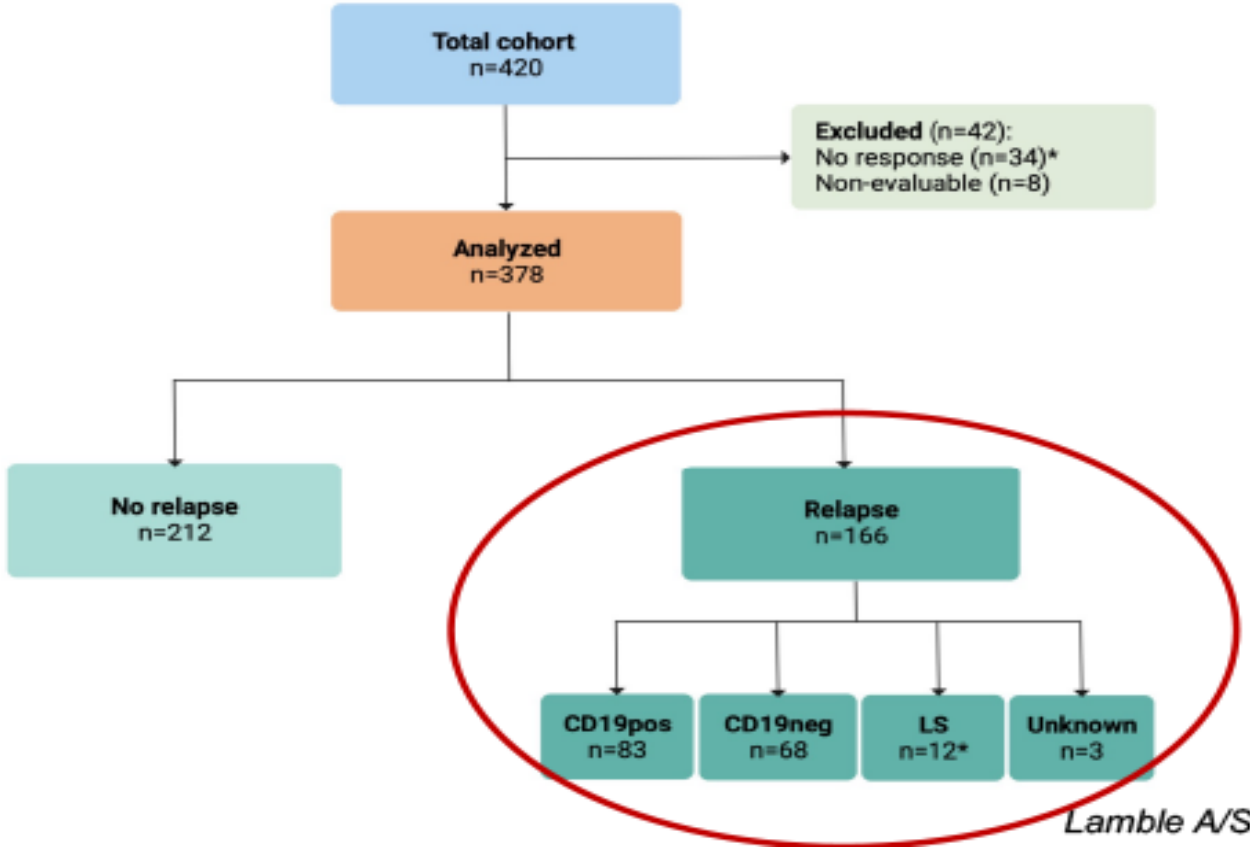
Etiology for relapse differs across the various phenotypic presentations



Lamble A/Shah NN, Blood Adv, 2022

Relapse

The nitty-gritty of relapse phenotype

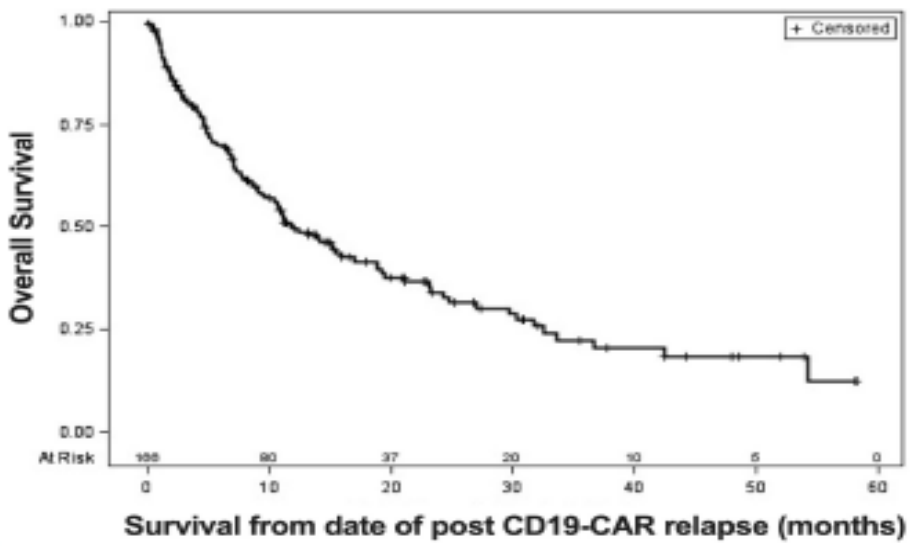


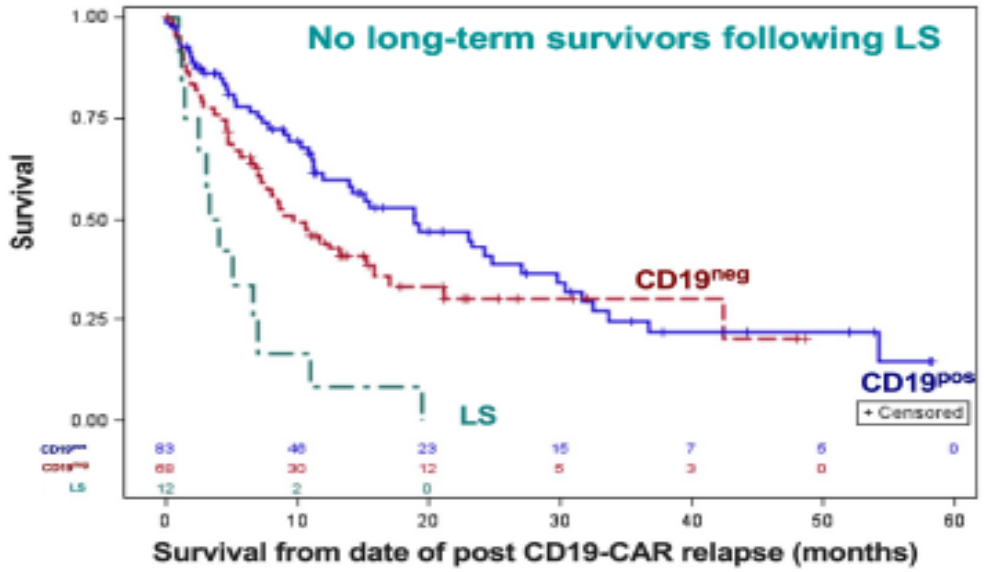
50% CD19+
41% CD19neg
7.2% LS
1.8% unknown

Lamble A/Shah NN, Blood Adv, 2022

Relapse phenotype

Relapse phenotype impacts outcomes





Lamble A/Shah NN, Blood Adv, 2022

Alternative antigen

Targeting an alternative antigen may help circumvent CD19 loss

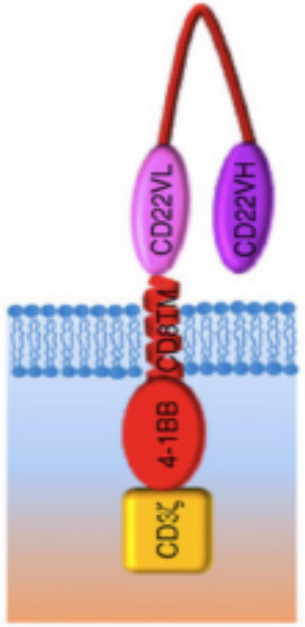
CD22 CAR T-cells

CD22 CAR T-Cells

- CD22 CAR:
  - m971 scFV
  - 4-1BB co-stimulatory domain

- CD22 CAR T-cells (NCI) highly active
  - Phase I, 3+3 dose escalation trial
  - NCI construct (m971/4-1BB)
  - CD22+ ALL or NHL
  - Ages 3-30 years
  - Lymphodepletion:
    - Fludarabine 75 mg/m^2 + Cyclophosphamide 900 mg/m^2
  - First patient infused December 2014
  - Now: 80 patients enrolled to date

Anti-CD22 CAR T-Cell

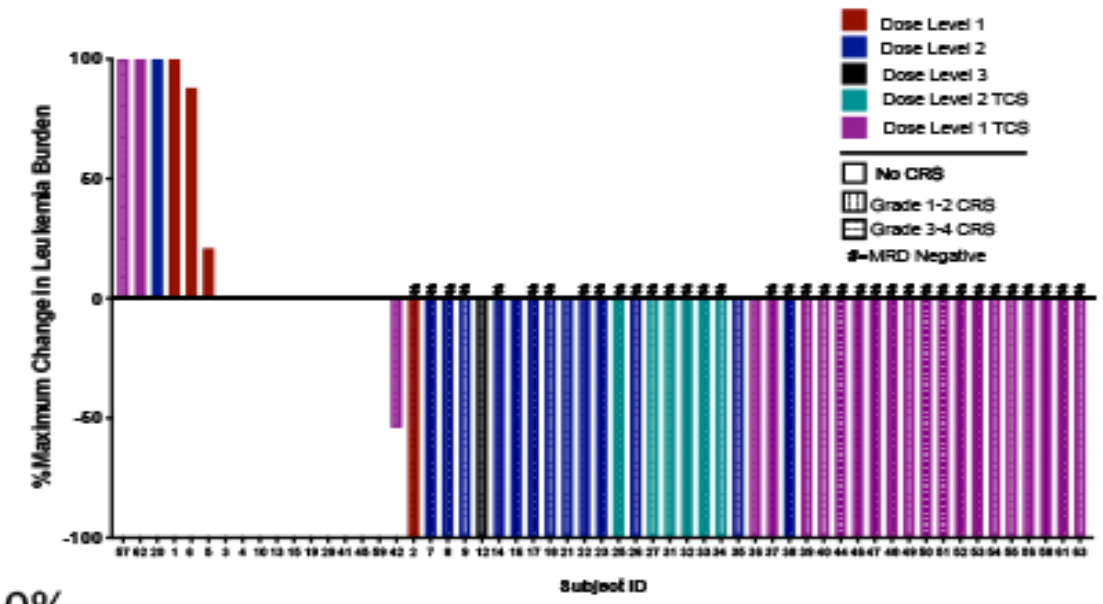


*Haso W, Lee DW, **Shah NN** et al. Blood 2013*

CD22 CAR T-cells are highly active

CD22 CAR T-cells are highly active in patients with relapsed/refractory disease

- **70% complete remission (CR) rate**
  - 40 of 58 patients
  - 87.5% minimal residual disease (MRD) negative
- **76% CR at expansion dose**
  - 19 of 25; MRD neg CR: 94.7%
- **Effective also in those who did not respond to prior CD19-targeted strategies**
- **Toxicity:**
  - Cytokine release syndrome grades 3/4: 10%
  - Neurotoxicity: mild

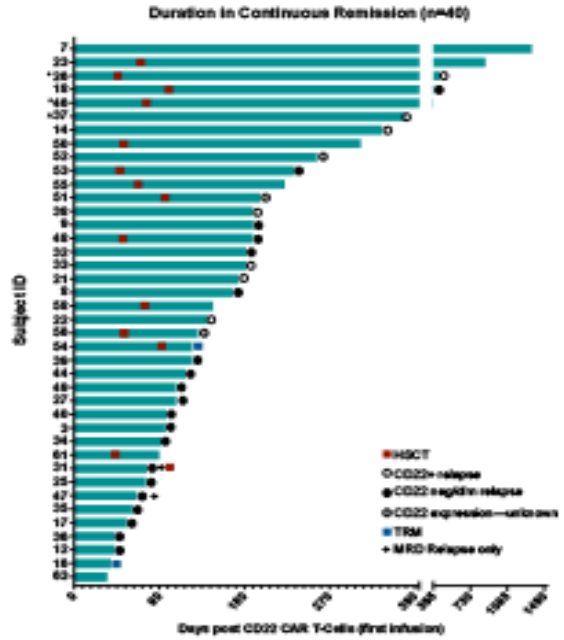


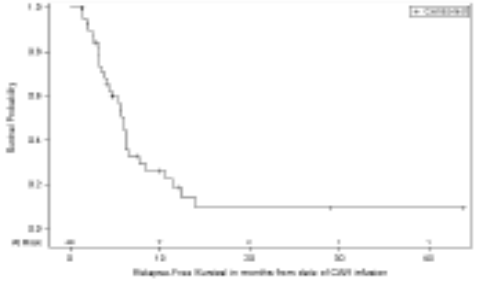
Shah et al., Journal of Clinical Oncology, 2020

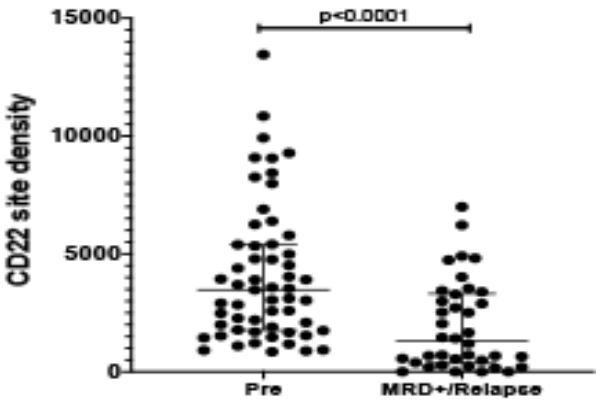
Remission reduction

Remission induction used as a bridge to HSCT to prevent antigen modulation as cause of relapse

- **Antigen modulation a frequent cause of relapse**
  - CD22 site density lower than CD19
- **AlloHSCT acceptable practice for curative intent in patients with r/r ALL**
  - Increased number of patients who have relapsed after CD19 CAR and not had a prior HSCT







Shah et al., Journal of Clinical Oncology, 2020

Breakthrough therapy

Breakthrough therapy designation

For the treatment of pediatric and young adult patients, 3-30 years of age with CD22 positive B-cell ALL that is refractory or in second or later relapse, and either CD19 negative or relapsed/refractory to CD19 targeting

https://ccr.cancer.gov/news/article/fda-grants-breakthrough-therapy-designation-for-new-car-t-cell-therapy-for-b-cell-acute-lymphoblastic-leukemia

FDA, August 2019

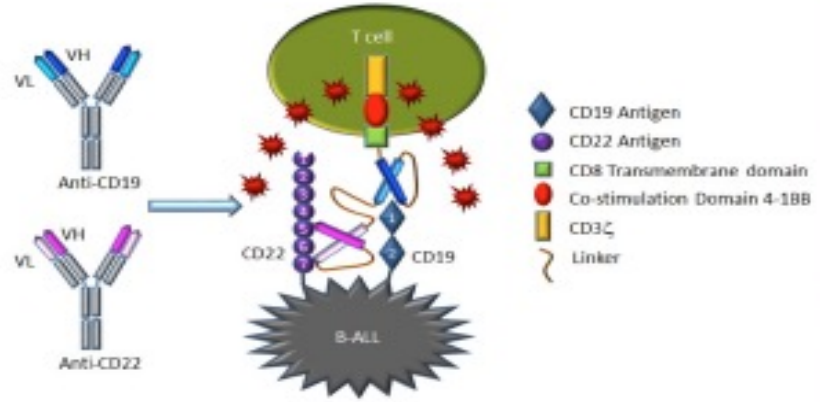
Antigen targeting strategies

Combinatorial/simultaneous antigen targeting strategies will be needed to prevent antigen escape

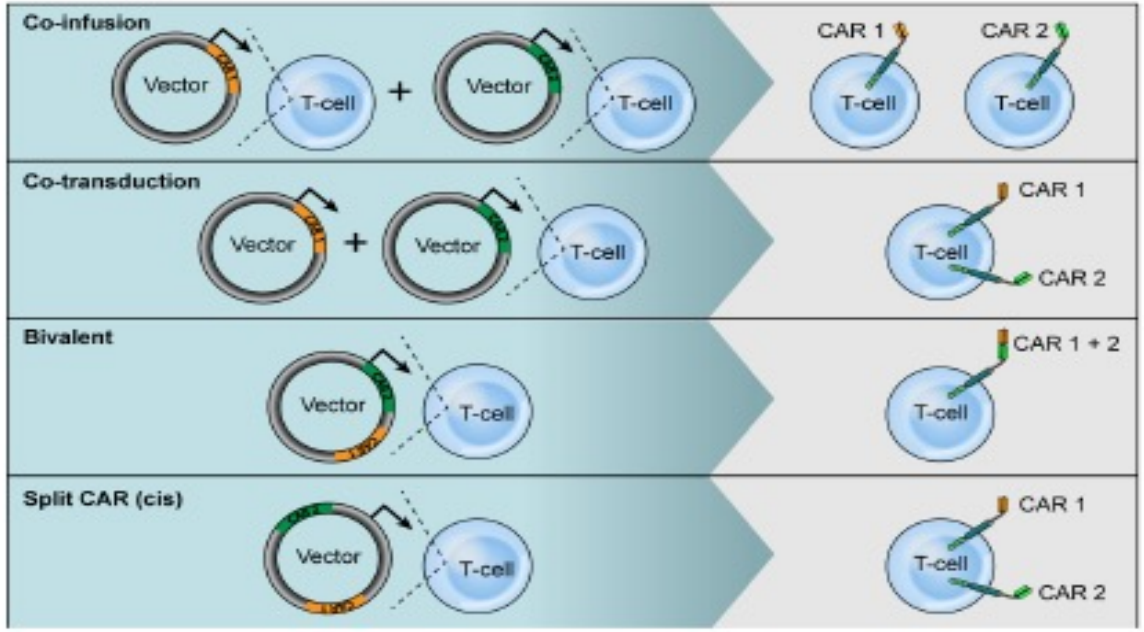
Combinatorial treatment strategies

The foundation of ALL therapy is based in combinatorial treatment strategies

- So… why would immunotherapy be any different?



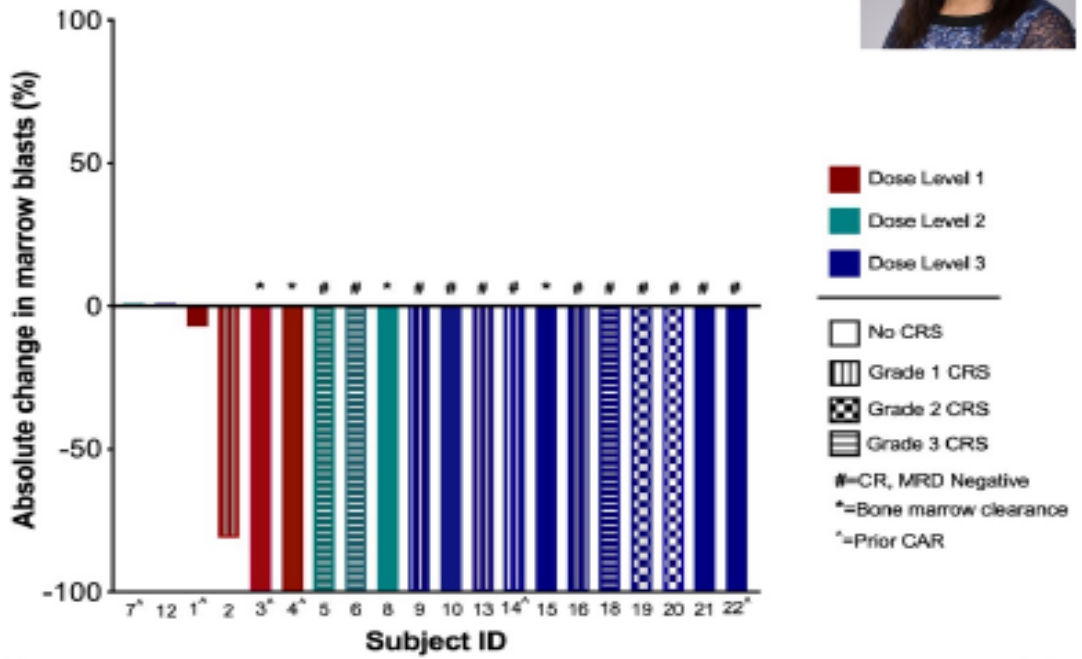
LoopCAR 6



Highly-active

Highly-active in r/r pre B-ALL

- 20 patients
  - 16 (80%) with eradication of marrow disease
  - 12 (60%) with complete CR
    - Discrepant responses in EMD
- Response was dose-dependent:
  - 14/16 (87.5%) at ≥ 1 x 10^6 transduced CAR T-cells
- CAR-naïve patients had improved response: 10/14
  - But CAR pre-treated patients also skewed towards the first (? Ineffective DL)
- CRS severity was generally low
  - 1 patient with ICANS (grade 3)
- Limited efficacy in extramedullary disease
- With limited CD22 targeting, a novel bicistronic construct will be forthcoming this summer



Shalabi H/Shah NN. Blood 2022

Stem cell transplantation

What is the role for allogeneic hematopoietic stem cell transplantation in CAR T-cell therapy?

HSCT

What is the role of HSCT following CD19 CAR T-cell therapy?

- CD19 targeted CAR T-cell therapy can lead to a long-term durable remission in a fraction of pediatric patients with B-ALL
- HSCT has an important role for consolidation and long-term cure in patients with high-risk or relapsed B-ALL
- HSCT is associated with both short-term and long-term risks
- Salvage options for patients relapsing after CD19 CAR T-cell therapy are limited

Prospective studies

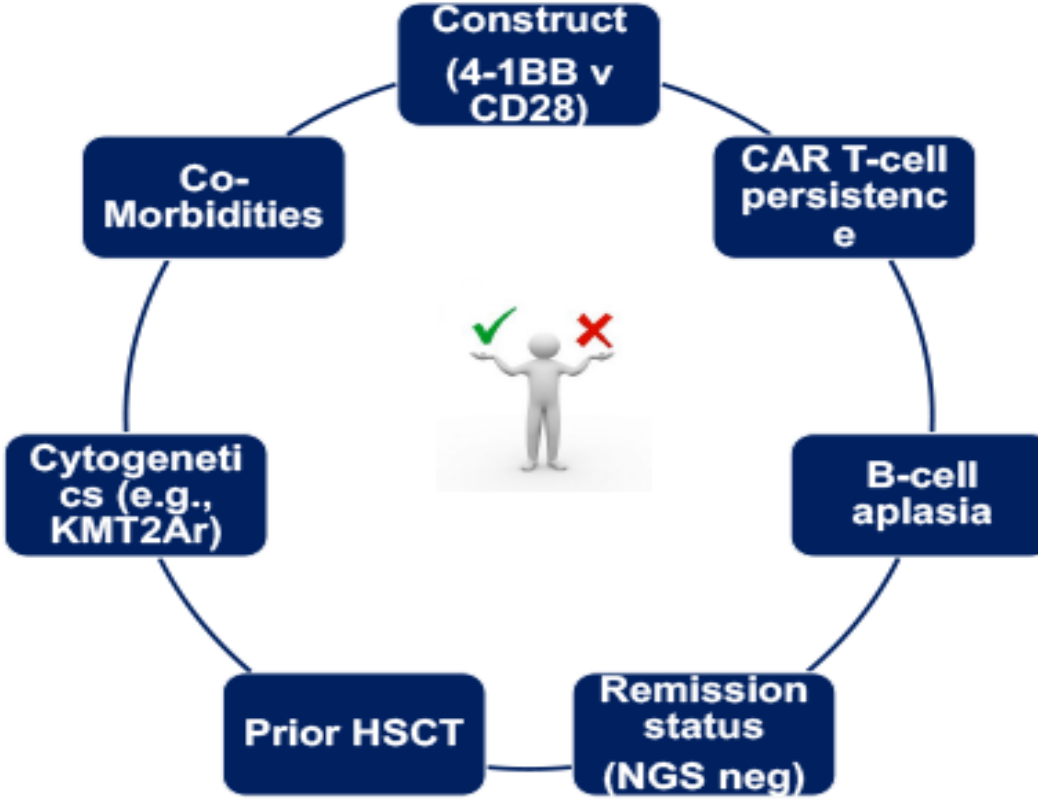
Prospective studies are needed to define the role for post-CAR consolidative HSCT

Benefit of HSCT

- NCI: CD19/28ζ
- Seattle: CD19/41BB
- MSK (peds): CD19/28ζ

No Benefit or Unknown

- Novartis/CHOP: CD19/41BB
- MSK (adult): CD19/28ζ



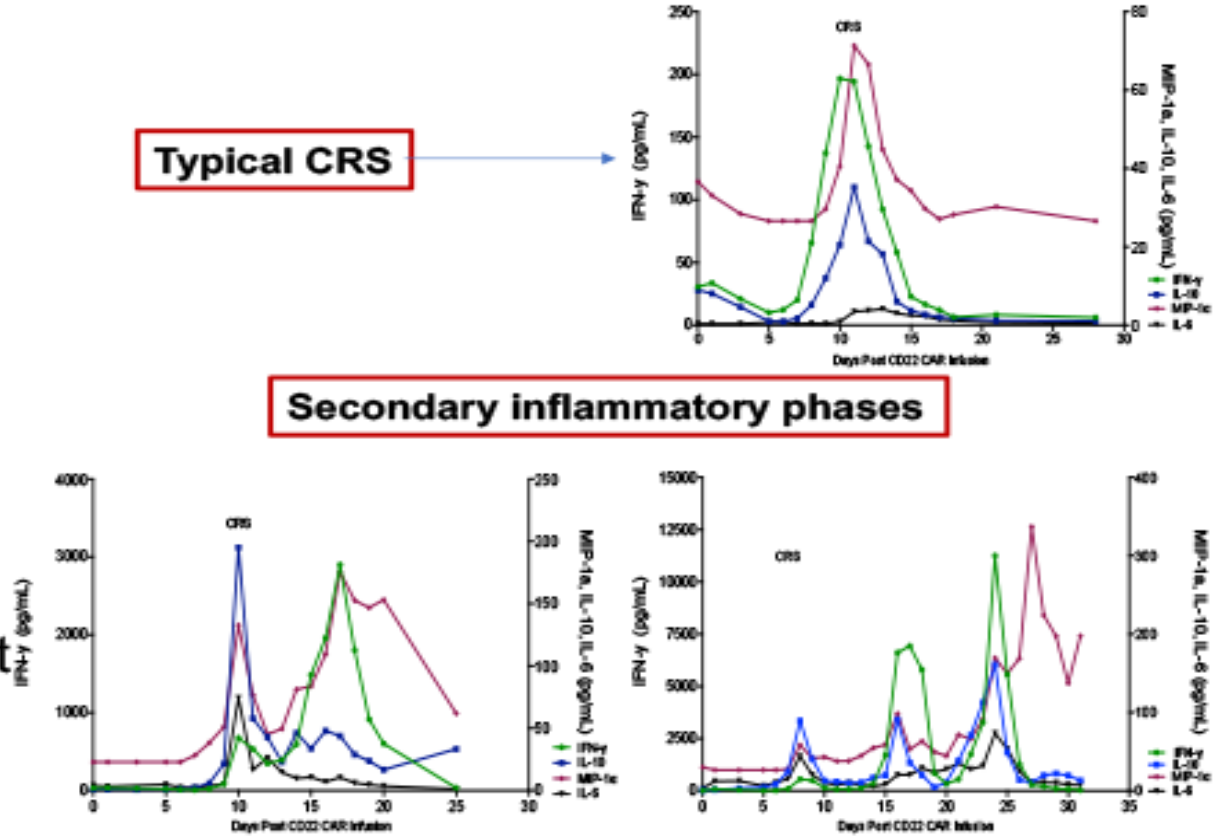
Novel toxicities

Novel toxicities will be seen with novel CAR T-cell constructs and targets

Secondary inflammatory phases

Secondary inflammatory phases seen in select patients treated with CD22 CAR T-cells

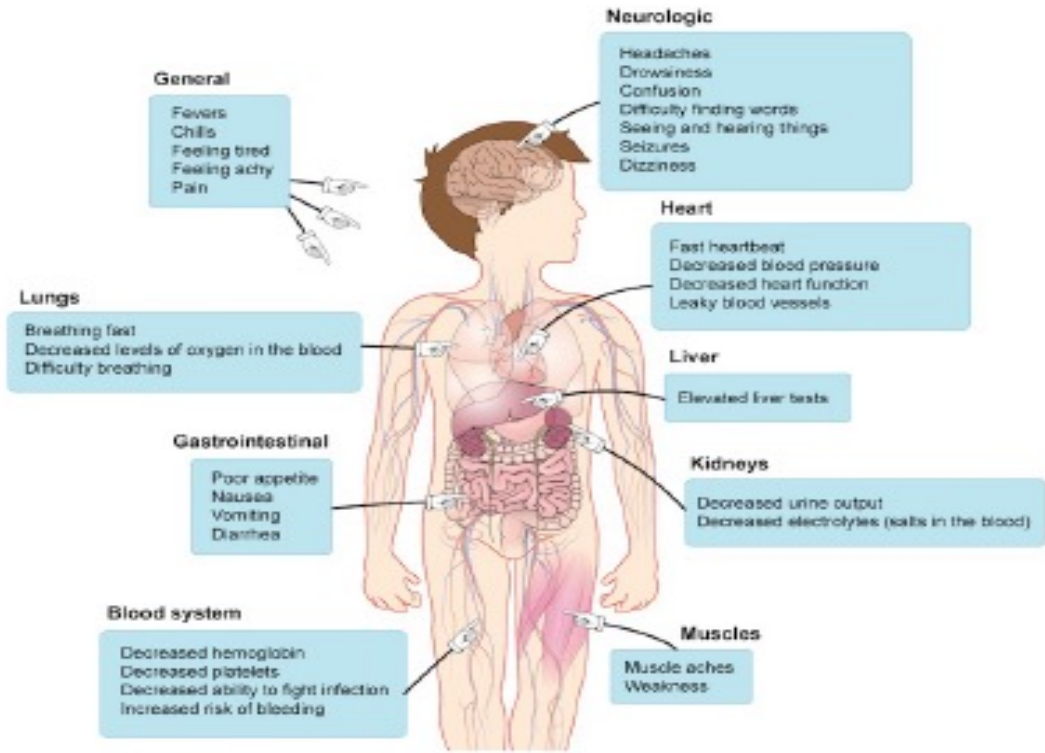
- Clinical manifestations
  - Cytopenias
  - Hepatic dysfunction
  - Elevated inflammatory markers (ferritin, sCD25),
  - Coagulopathy (hypofibrinogenemia)
- Symptoms often occur after clinical resolution from CRS
- Indications for tocilizumab administration often were not met during 2° symptoms



Cytokine release syndrome

Cytokine Release Syndrome

- Supraphysiologic inflammatory process seen with CAR T-cell expansion
- Range from mild to severe (life-threatening)
- Neurotoxicity particularly worrisome
- Tocilizumab (anti-IL6 receptor Ab) FDA approved for the treatment of CRS



Secondary phases

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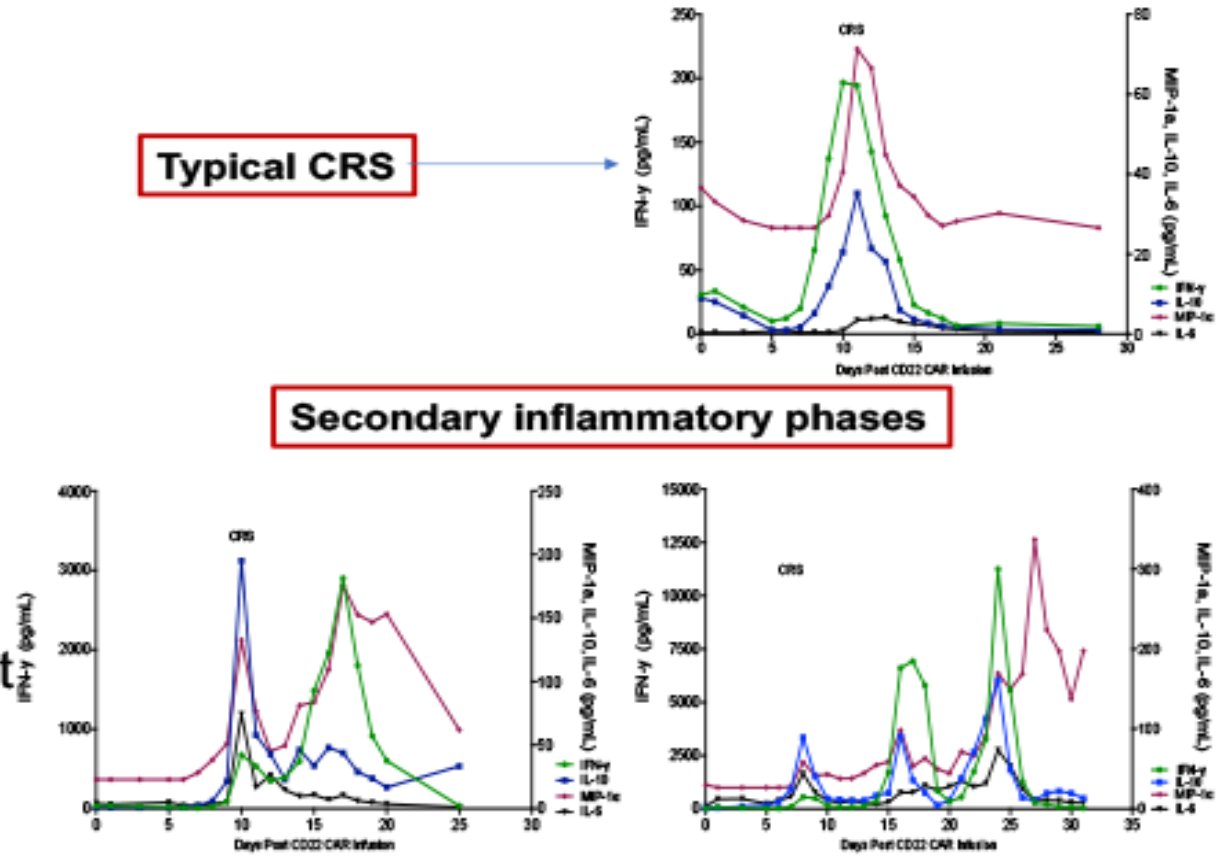


Immune effector cell

Immune Effector Cell associated HLH-like Syndrome (IEC-HS)

Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome

Melissa R. Hines[1], Tristan E. Knight[2], Kevin O. McNerney[3], Mark B. Leick[4], Tania Jain[5], Sairah Ahmed[6], Matthew J. Frigault[4], Joshua A. Hill[7], Michael D. Jain[8], William T. Johnson[9], Yi Lin[10], Kris M. Mahadeo[11], Gabriela M. Maron[12], Rebecca A. Marsh[13], Sattva S. Neelapu[6], Sarah Nikiforow[14], Amanda K. Ombrello[15], Nirav N. Shah[16], Aimee C. Talleur[17], David Turicek[18], Anant Vatsayan[19], Sandy W. Wong[20], Marcela V. Maus[4], Krishna V. Komanduri[20], Nancy Berliner[21], Jan-Inge Henter[22], Miguel-Angel Perales[23], Noelle V. Frey[24], David T. Teachey[25], Matthew J. Frank[26], Nirali N. Shah[18,*]

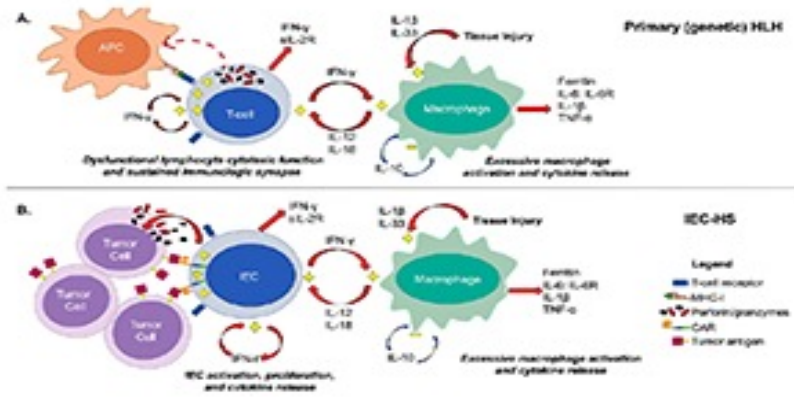


Table 1
IEC-HS: Definition and Identification

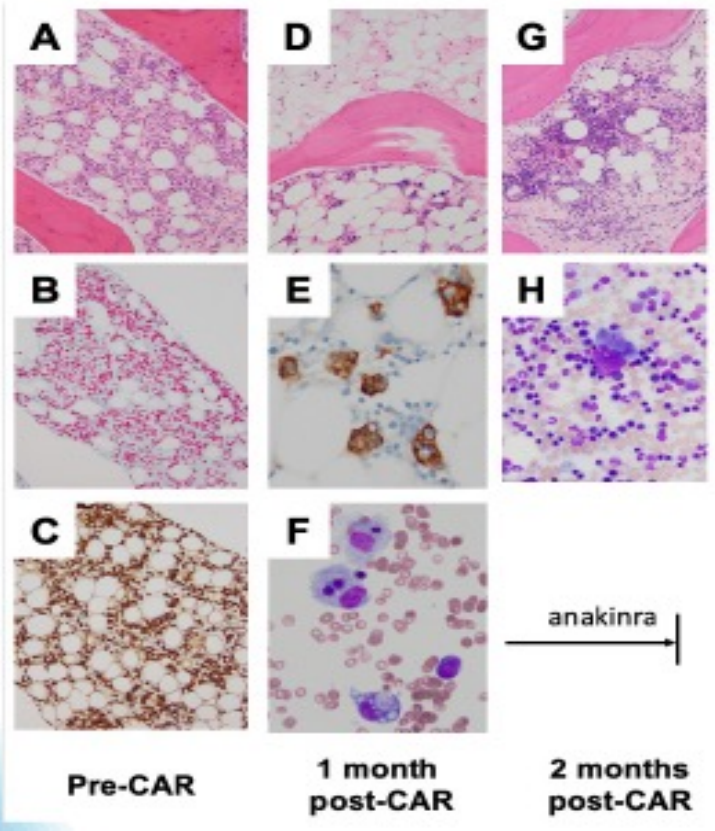
Definition of IEC-HS	The development of a pathological and biochemical hyperinflammatory syndrome independent from CRS and ICANS that (1) manifests with features of macrophage activation/HLH, (2) is attributable to IEC therapy, and (3) is associated with progression or new onset of cytopenias, hyperferritinemia, coagulopathy with hypofibrinogenemia, and/or transaminitis
Criteria for identifying IEC-HS[†]	**Clinical/Laboratory Manifestations**
Most common manifestations[‡]	Required: elevated ferritin (≥2 × ULN or baseline [at time of infusion][§] and/or rapidly rising [per clinical assessment])
	Onset with resolving/resolved CRS or worsening inflammatory response after initial improvement with CRS-directed therapy[‖]
	Hepatic transaminase elevation[¶] (≥5 × ULN [if baseline was normal] or ≥5 × baseline if baseline was abnormal)
	Hypofibrinogenemia (≤150 mg/dL or <ULN)[#]
	Hemophagocytosis in bone marrow or other tissue[‡]
	Cytopenias (new onset, worsening, or refractory[**])
Other manifestations that may be present	Lactate dehydrogenase elevations (>ULN)
	Other coagulation abnormalities (eg, elevated PT/PTT)
	Direct hyperbilirubinemia
	New-onset splenomegaly
	Fever (new[‖] or persistent)[‡]
	Neurotoxicity
	Pulmonary manifestations (eg, hypoxia, pulmonary infiltrates, pulmonary edema)
	Renal insufficiency (new onset)
	Hypertriglyceridemia (fasting level, ≥265 mg/dL[††])

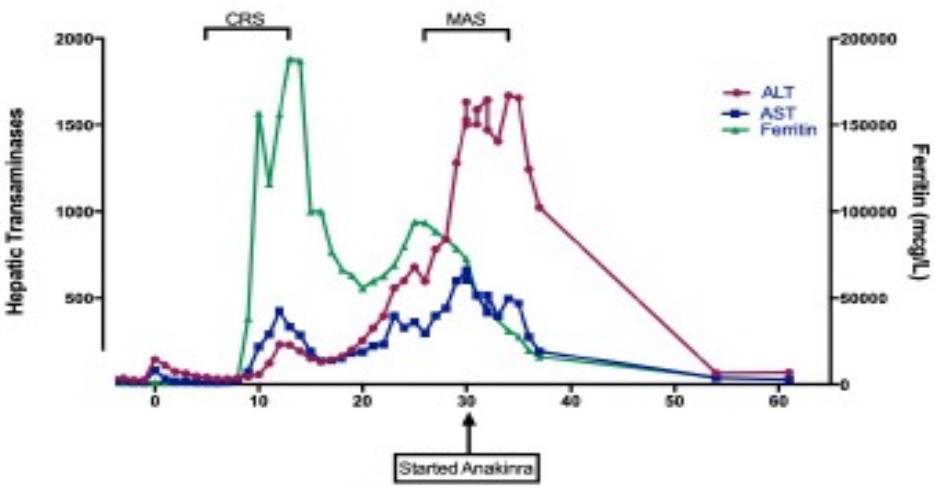
ULN indicates upper limit of normal; LLN, lower limit of normal.
* Diagnosis was made only when not attributable to alternative etiologies, including CRS, infection and/or disease progression.
† Constellation of findings typically simultaneously (eg, all within 72 hours).
‡ Although most cases of IEC-HS have been seen with antecedent CRS, this may not always be the case, and emerging experience will shed light on how IEC-HS may present.
§ Consistent with grade 3 hepatic transaminase elevations according to Common Terminology for Adverse Events version 5.0.
‖ According to HLH-2004.
¶ Generally at least 1 lineage will be a grade 4 cytopenia (platelets, neutrophils, hemoglobin).
# As distinguished from CRS onset or recrudescence.

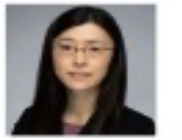
Hines/Shah, Transplantation and Cellular Therapy, 2023

Novel toxicities

Novel toxicities necessitate unique approaches: Anakinra targeting of IL-1 signaling reduced carHLH symptoms







POB CRS guidelines updated to incorporate utilization of anakinra in treatment of HLH-like toxicities of CRS

Shah et al., Journal of Clinical Oncology, 2020

Beyond ALL

Going beyond ALL…

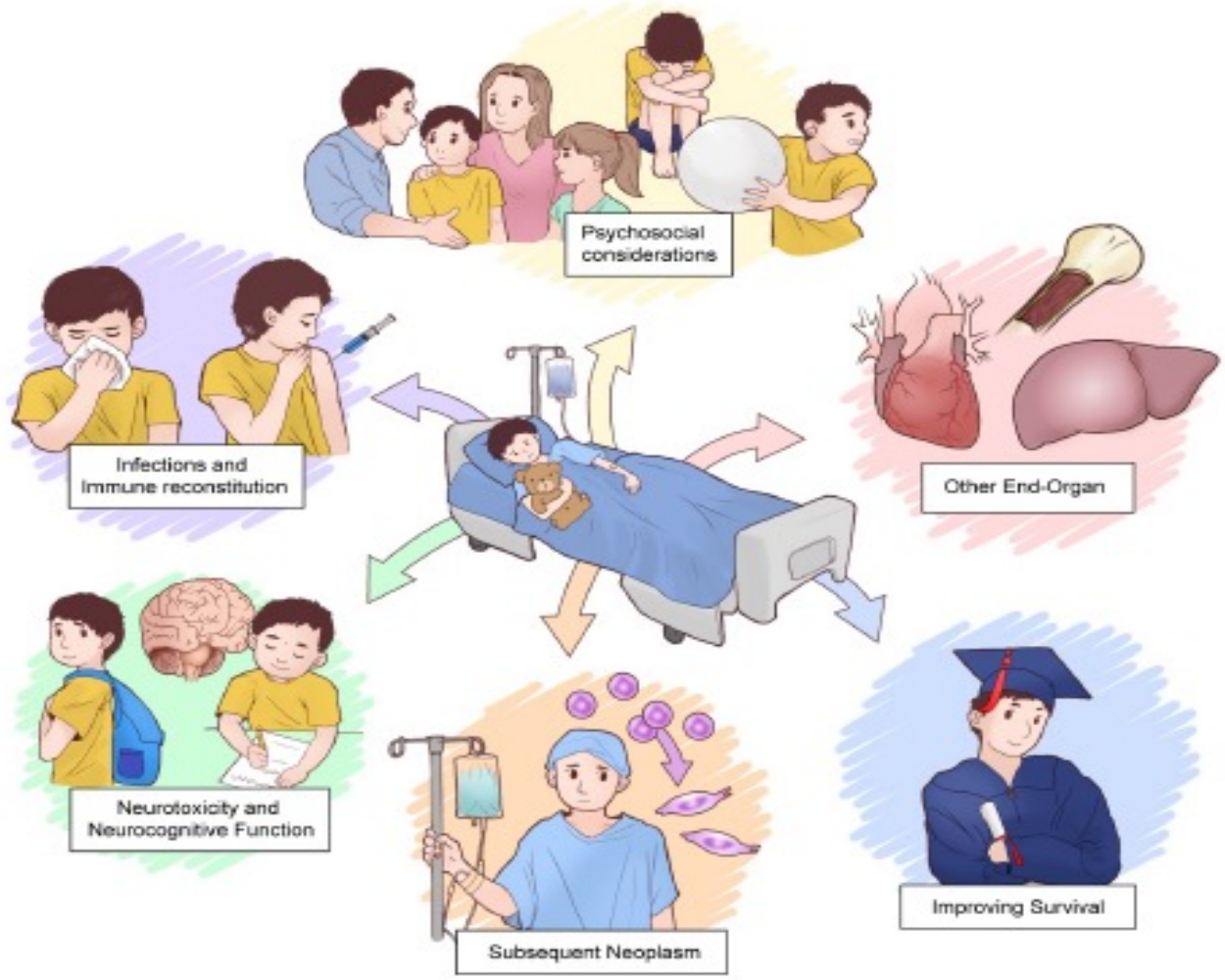
- 60% of children and young adults with AML will not achieve long-term durable remission
- CD33 is an established target for AML
  - CD33 CAR construct developed in the POB (Qin/Fry)
- Phase I dose escalation study of CD33 CAR T-cells in children and young adults with r/r AML
  - Bridge to HSCT given concern for CD33 expression on hematopoietic precursors
- Trial updates:
  - First multicenter phase 1 CAR T-cell trial where manufacturing was done at NCI Frederick
  - Dose level 1 completed, 3 patients treated to date

Acute effects

What do we know about subacute or other long-term effects?

Beyond the storm

CAR T-cell Therapy: Beyond the Storm



https://ncifrederick.cancer.gov/events/conferences/car-t-cell-therapy-beyond-storm

Consortium

Beyond the Storm consortium

- Multi-center, multi-disciplinary group of care providers who are all well-versed in early implementation of CAR T-cell therapy

- Retrospective/prospective protocols to study subacute/late effects of CAR T-cell toxicities in children and young adults

Future directions

Areas of active research and future directions

- CAR T-cell highly effective in B-cell malignancies, however opportunities to further optimize this strategy remain
  - Relapse treatment and prevention
    - Antigen modulation
  - Toxicity management (acute and late effects)
  - Extending this therapy to other diseases
  - CAR T-cell manufacturing and design
  - Timing

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