Gene Therapy in Oncology: Past, Present and Future

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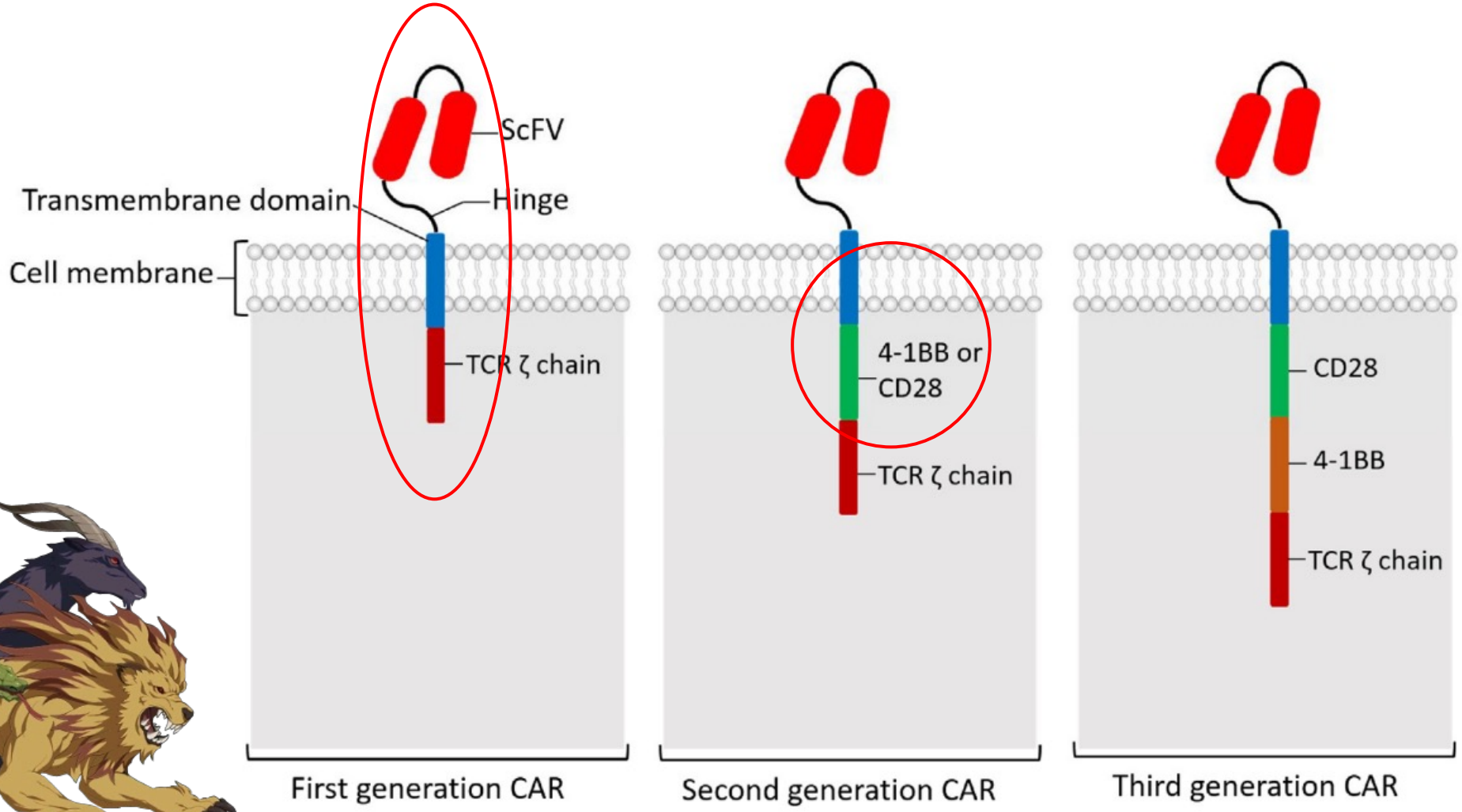
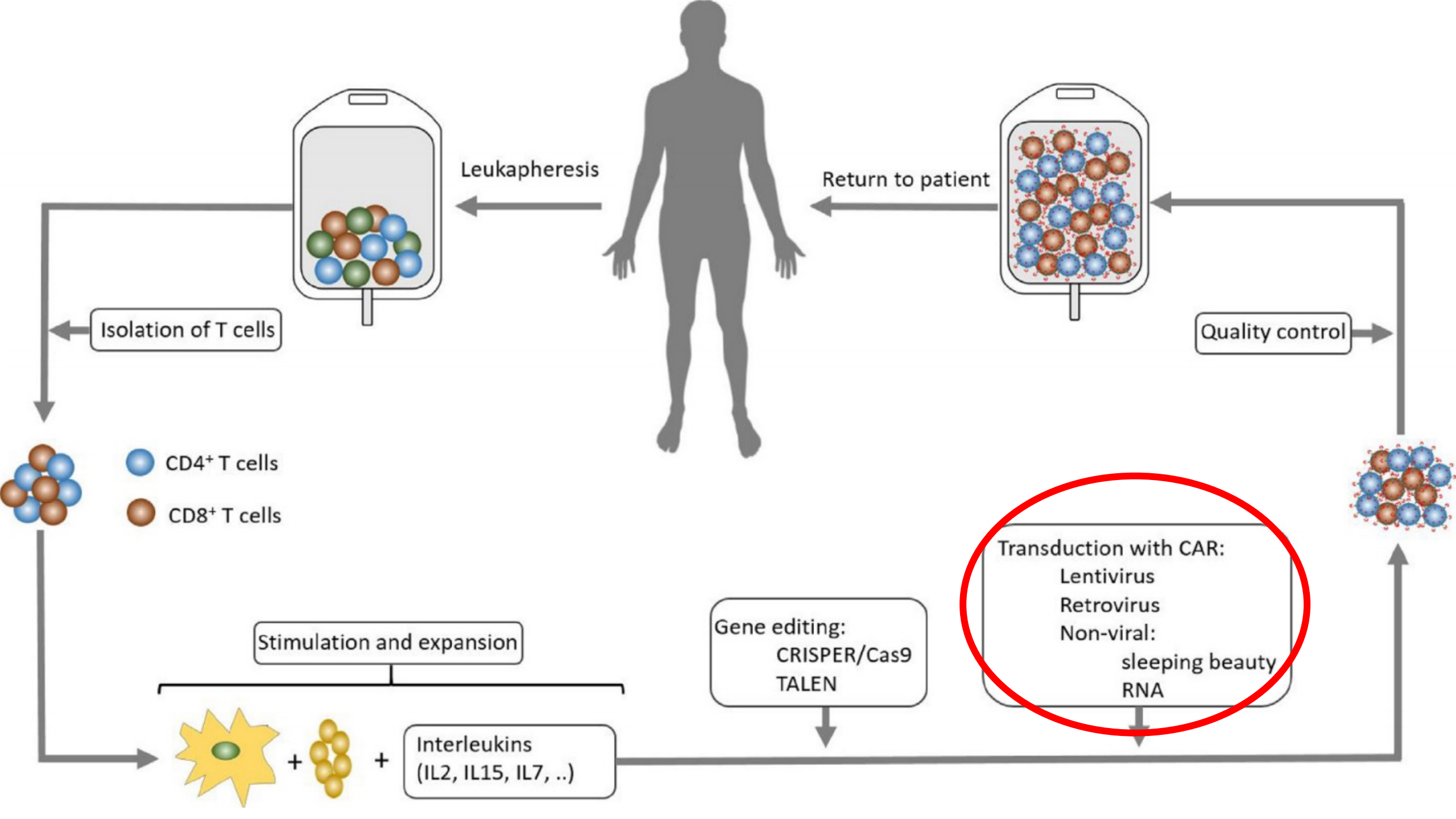
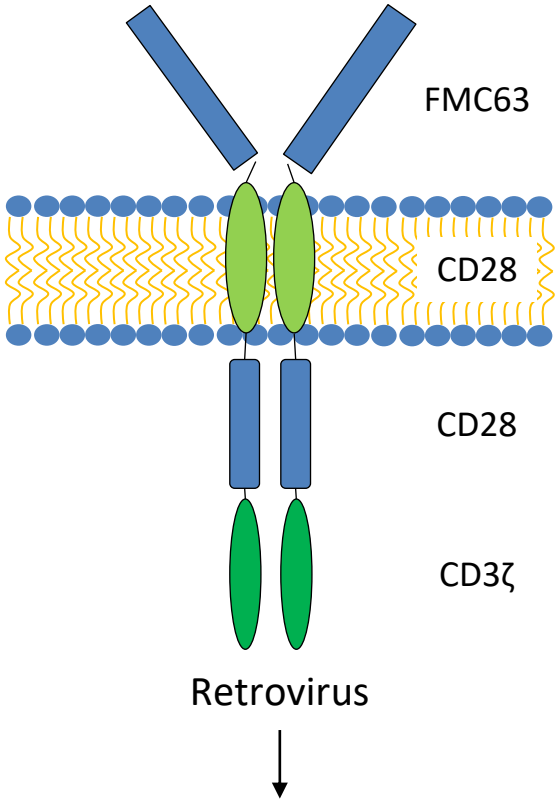
Chimeric Antigen Receptor or CAR-T Cell



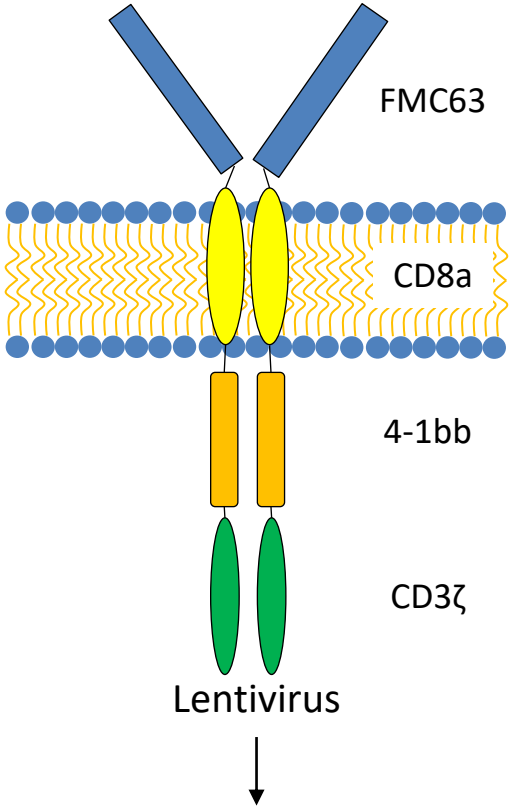

Leukapheresis
Return to patient
Isolation of T cells
Quality control
CD4+ T cells
CD8+ T cells
Transduction with CAR:
Lentivirus
Retrovirus
Non-viral:
sleeping beauty
RNA
Gene editing:
CRISPER/Cas9
TALEN
Stimulation and expansion
+
+
Interleukins
(IL2, IL15, IL7, ..)

Three Anti-CD19 CAR T-cell Products are FDA Approved as 3rd Line Therapy for Rel/Ref DLBCL

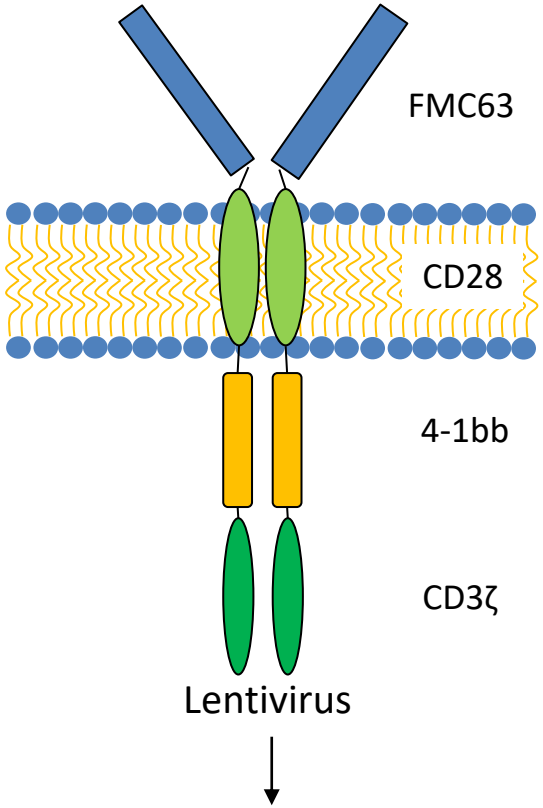
Axicabtagene ciloleucel
(*aka* Axi-cel; Yescarta)
NCI



Tisagenlecleucel
(*aka* Kymriah)
UPenn



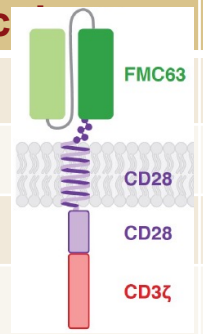
Lisocabtagene maraleucel
(*aka* Liso-cel)
FHCR

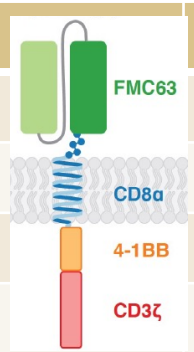


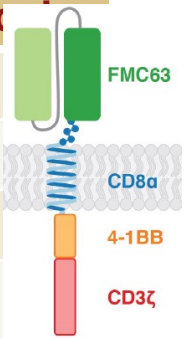
[1] Adapted from: van der Stegen SJ et al. Nat Rev Drug Discov. 2015 Jul;14(7):499-509.

Three Major Anti-CD19 CAR T-Cell Products for Aggressive B-Cell NHL

	Axicabtagene Ciloleucel	Tisagenlecleucel	Lisocabtagene Maraleucel
Construct	antiCD19-**CD28**-CD3z	antiCD19-**41BB**-CD3z	antiCD19-**41BB**-CD3z
Vector	Retrovirus	Lentivirus	Lentivirus
T-cell manufacturing	Bulk	Bulk	Defined doses CD4, CD8
Dose	2 × 10^6/kg (max 2 x 10^8)	0.6 to 6.0 x 10^8	DL1: 0.5 x 10^7 DL2: 1.0 x 10^8 DL3: 1.5 x 10^8
Bridging therapy	None allowed in pivotal trial but often used in standard practice	93%	72%
Lymphodepletion	Flu/Cy 500/30 x 3d	Flu/Cy 250/25 x 3d, or Benda	Flu/Cy 300/30 x 3d
Approval status	FDA/EMA approved for DLBCL, high grade B-cell lymphoma, transformed FL, PMBCL	FDA/EMA approved for pediatric B-ALL, DLBCL, high grade B-cell lymphoma, transformed FL	Not yet FDA/EMA approved
Best ORR	83%	52%	73%
Best CR	58%	40%	53%







Locke FL, et al. Lancet Oncol. 2019.
Schuster SJ, et al. N Engl J Med. 2019.
Abramson JS, et al. Lancet 2020.

ZUMA-1: Axi-cel in r/r large B-cell lymphoma

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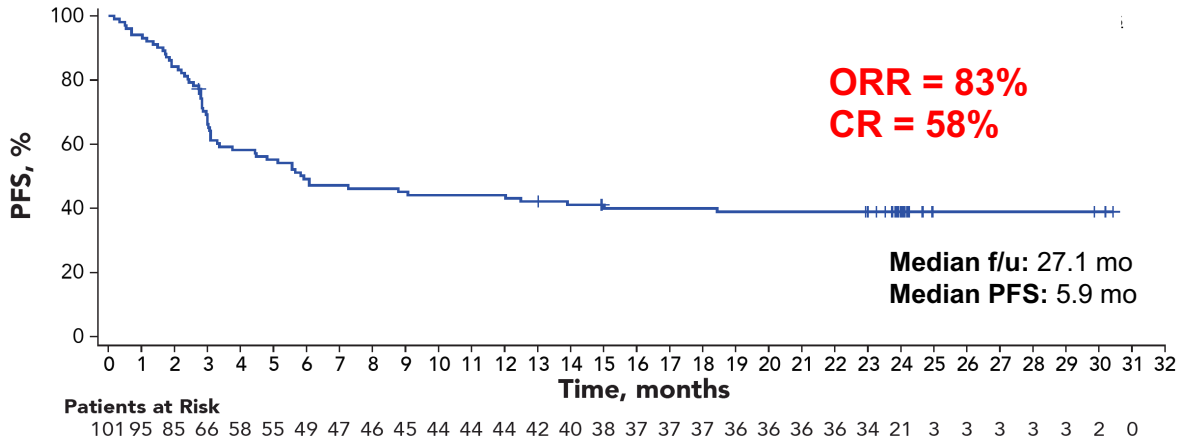
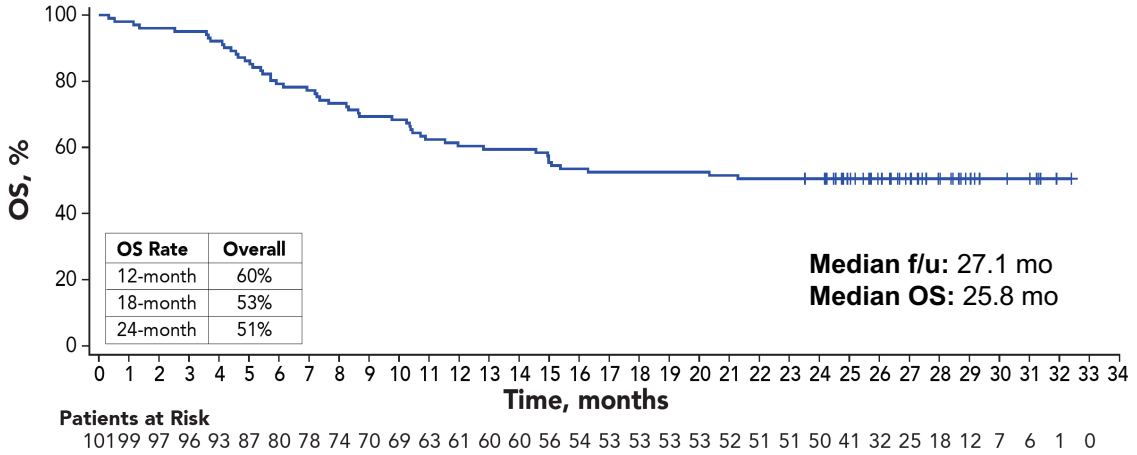
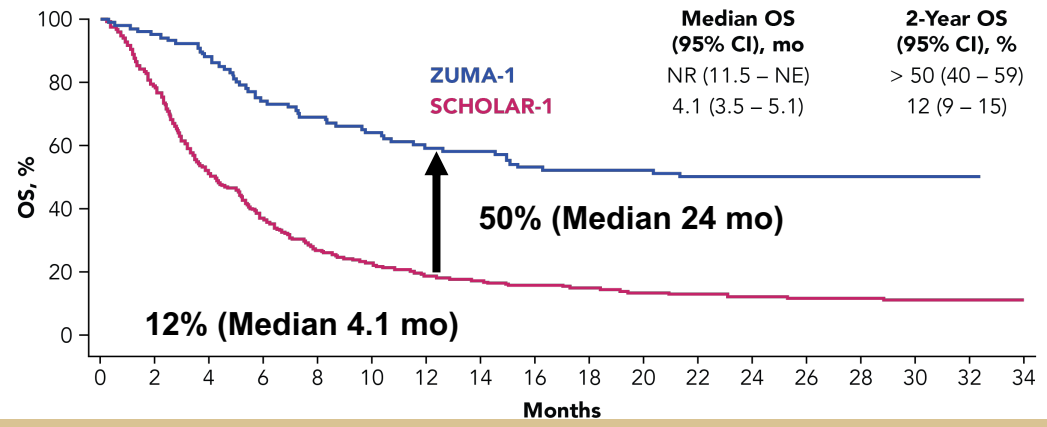
PFS: 39% progression-free at 27.1 mo

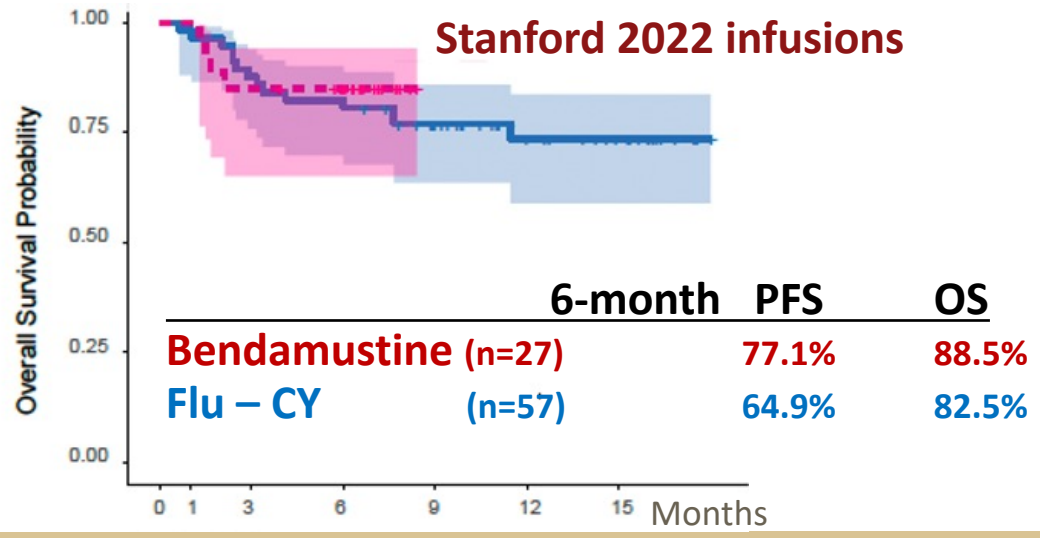


OS: 51% alive at 27.1 mo



OS Comparison: ZUMA-1 vs. SCHOLAR-1



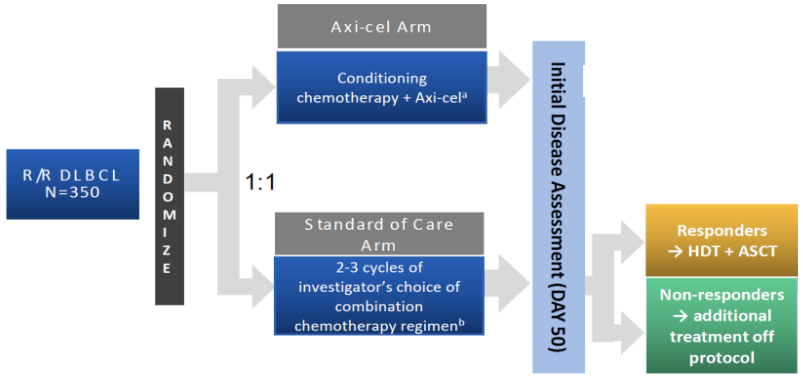


Pivotal Trials for LBCL in 2L- Is EFS Superior with CART Vs SOC followed by ASCT?

ZUMA 7

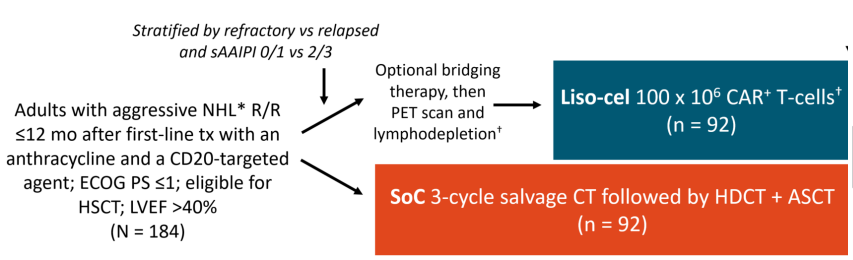
ORIGINAL ARTICLE

Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma



TRANSFORM

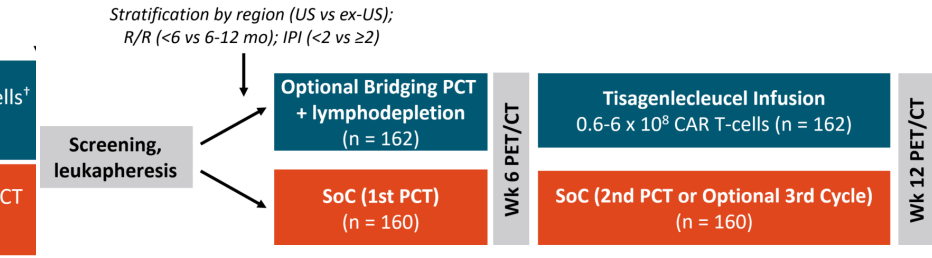
Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial



BELINDA

ORIGINAL ARTICLE

Second-Line Tisagenlecleucel or Standard Care in Aggressive B-Cell Lymphoma



Primary Endpoint	**EFS**	**EFS**	**EFS**
N	**350**	**184**	**322**
Vein to vein time (product available from apheresis)	**26 days (18 days)**	**36 days (26 days)**	**52 days (n.a)**

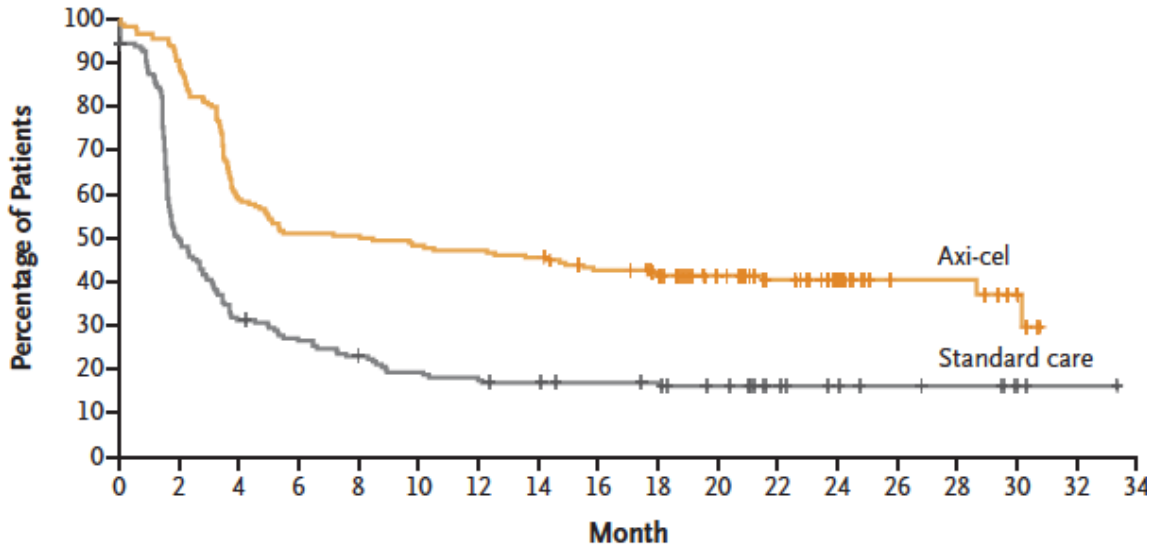
Locke, Miklos NEJM 2021

Kamdar et al, Lancet, 2022

Bishop et al. NEJM 2021

Event Free Survival in 2nd line Randomized trials Superior With CART Therapy

ZUMA-7 Axi-Cel



Median EFS= 8.3 months (vs 2.0m)

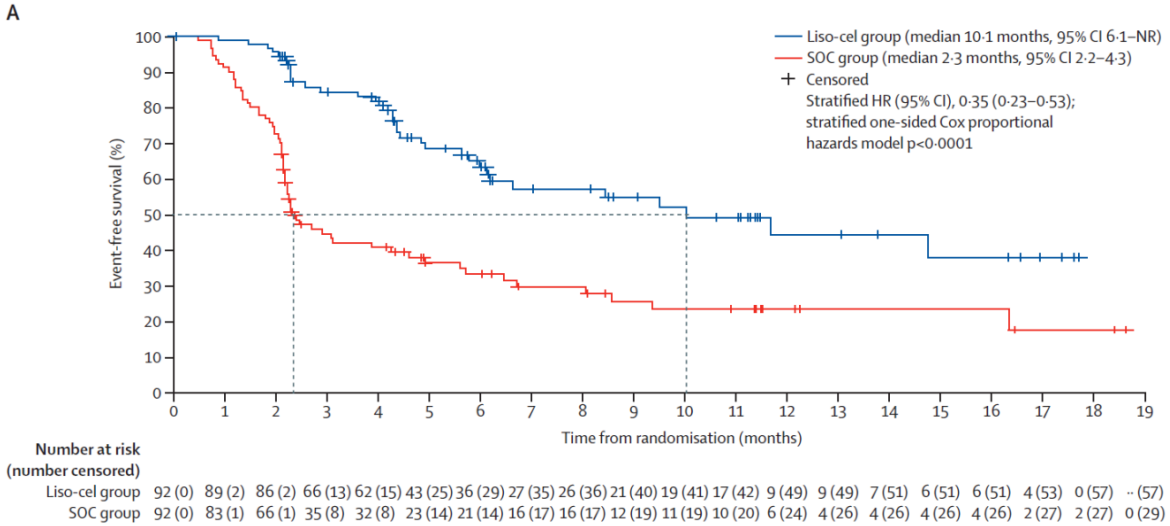
2-year EFS= 41% (vs 16%)

Grade 3/4 CRS= 6%

Grade 3/4 ICANS= 22% (elevated LDH 56%)

Locke, Miklos.. NEJM 2021.

TRANSFORM Liso-Cel



Median EFS= 10.1 months (vs 2.3m)

1-year EFS= 44% (vs 20%)

Grade 3/4 CRS= 1%

Grade 3/4 ICANS= 4% (elevated LDH 11%)

Kamdar et al, Lancet, 2022

ZUMA-7: Axi-cel more than doubled the number of patients who remained event-free at 2 years vs. SOC

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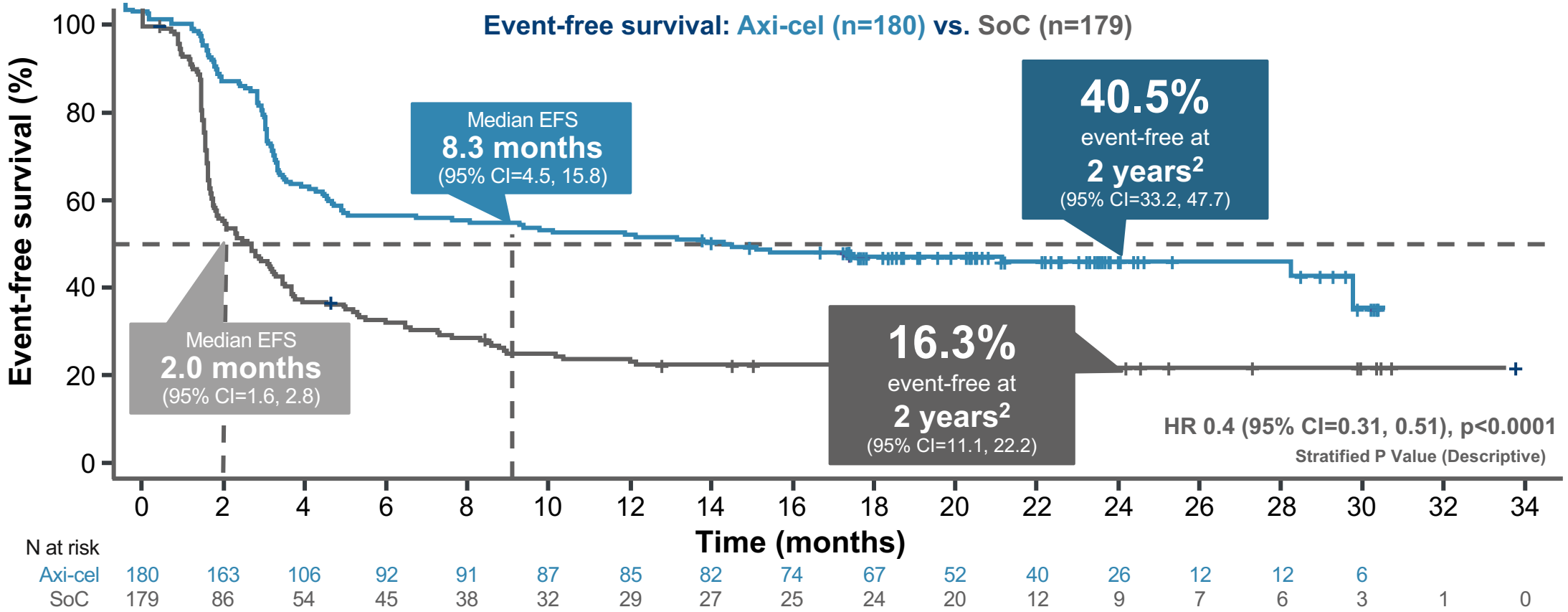

There was a 60% reduction in the risk of events with axi-cel vs. SoC in patients with R/R DLBCL

CI: confidence interval; HR: hazard ratio; mFU: median follow-up
Westin JR, *et al*. ASCO 2022 (Abstract 7548; poster).

CAR T-Cell Persistence and B-Cell Recovery

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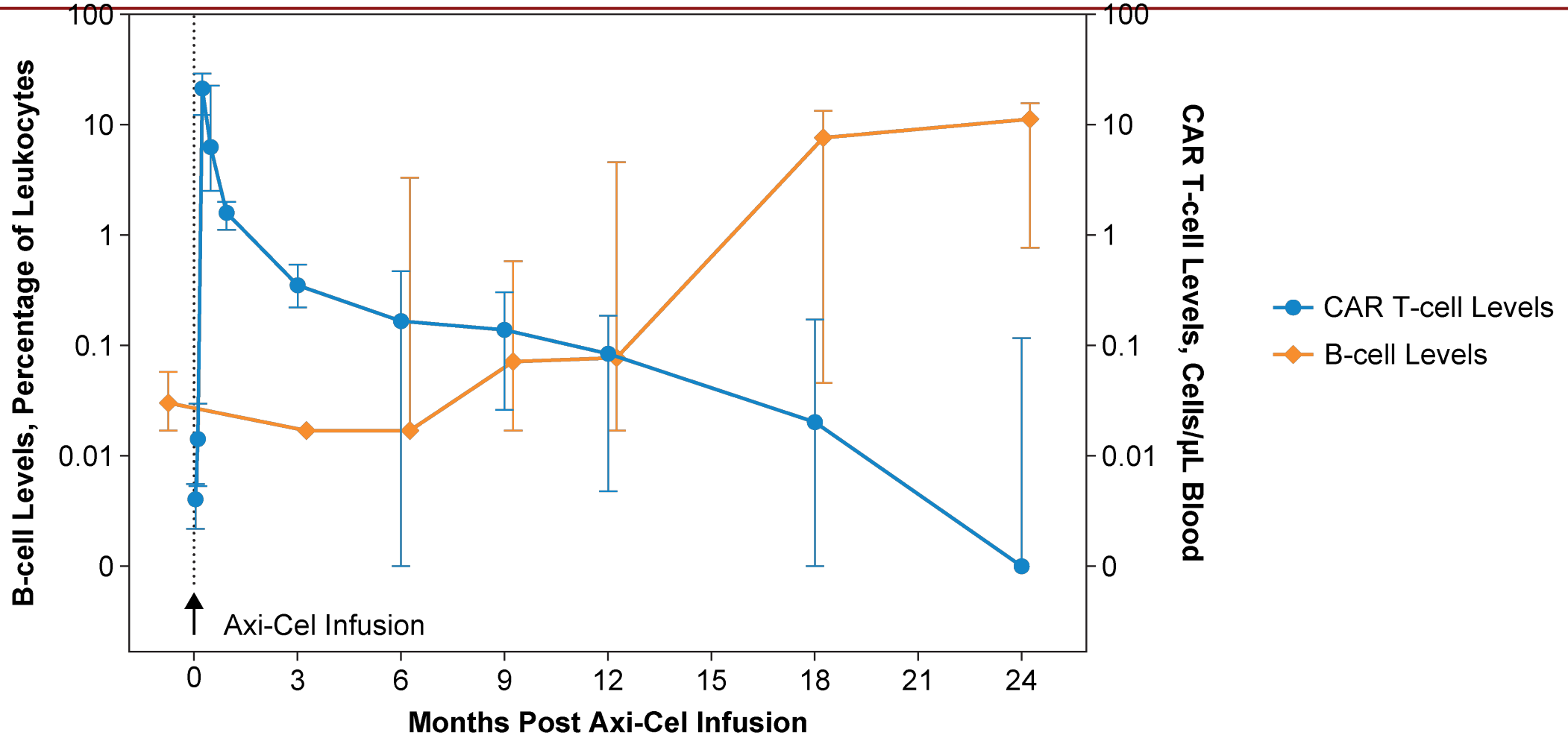


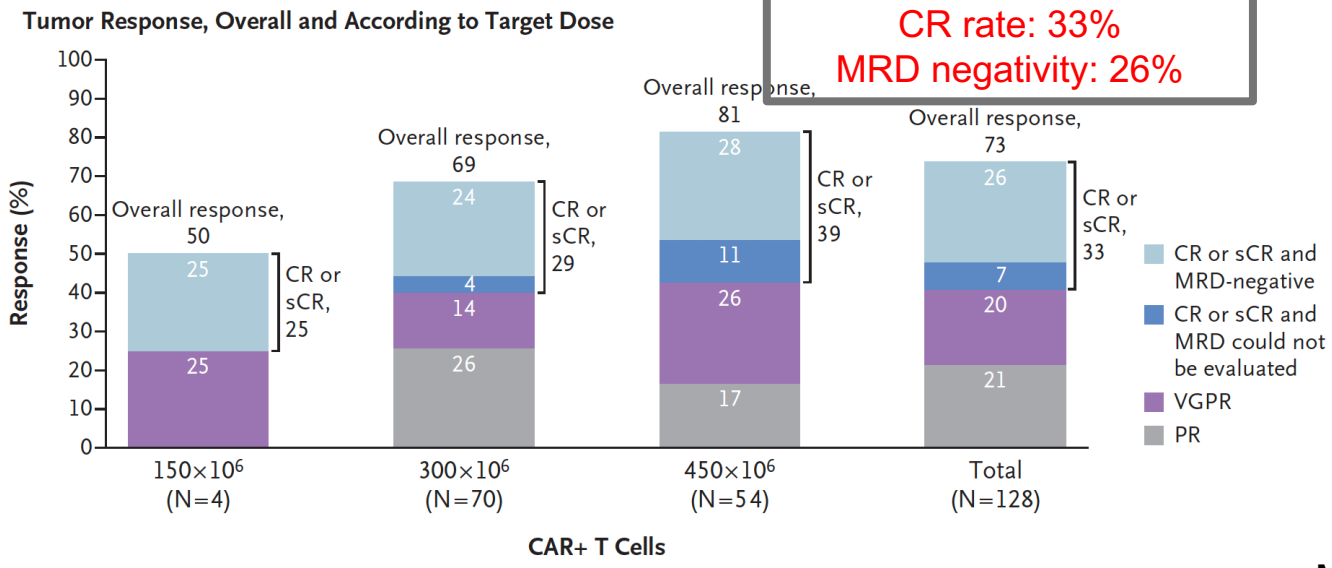
Idecabtagene Vicleucel (Ide-cel): FDA Approved March 2021

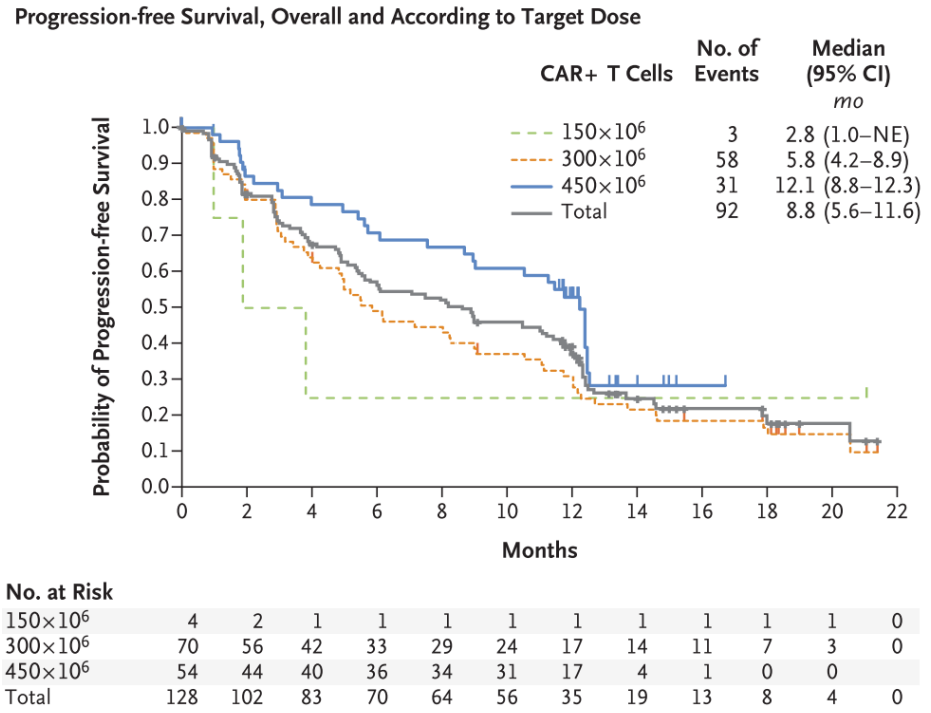
Baseline Characteristics	N=128
Median age	61 years
Target dose	300-450 million
Median Prior Lines	6
Triple Class Refractory	84%
Penta Refractory	26%
Bridging Therapy	88%

Overall response rate: 73%

CR rate: 33%

MRD negativity: 26%





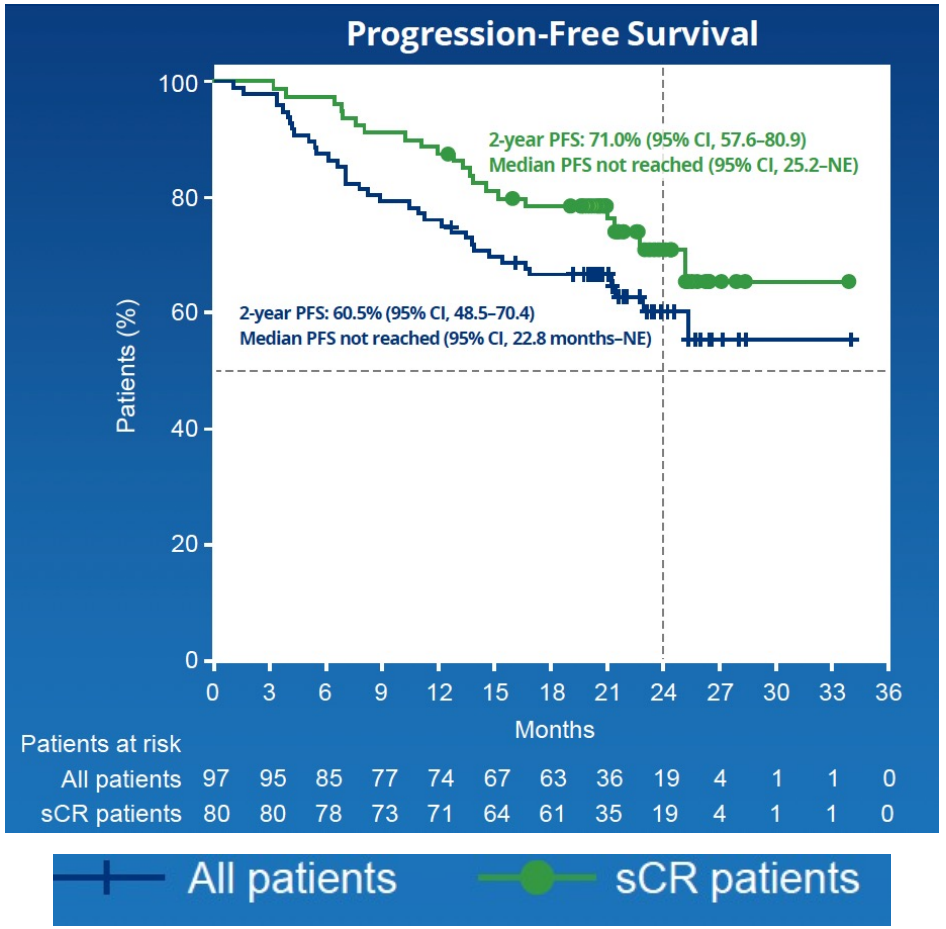
Survival Outcomes	
Median PFS	8.8 months
Median PFS in CR	20.2 months
Median OS	19.4 months

Munshi et al. NEJM 2021;384(8):705-716

Ciltacabtagene Autoleucel (Cilta-cel): FDA Approved March 2022

Baseline Features	
N	97
Target CAR-T Dose	0.75 million/kg
Median age	61 years
Median prior lines	6
Triple Class Refractory	88%
Penta Refractory	42%

Efficacy	
ORR	98%
sCR rate	83%
MRD negative rate (10^{-5})	58%[2]
PFS	2 year: 61%, median NR
OS	2 year: 74%, median NR



1. Martin et al. ASH 2021 *Blood* (2021) 138 (Supplement 1): 549.
2. Usmani et al ASCO 2021. JCO 2021;39(15_suppl):8005.

FDA-Approved CAR T-Cell Therapies

THERAPY	INDICATIONS
CD19-Targeting Therapies	
Axicabtagene ciloleucel	▪ **Adults with large B-cell lymphoma refractory to or relapsed within 12 mo of first-line chemoimmunotherapy** ▪ **Adults with R/R large B-cell lymphoma after ≥2 lines of systemic therapy,** including DLBCL NOS, DLBCL arising from follicular lymphoma, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma ▪ **Adults with R/R follicular lymphoma after ≥2 lines of systemic therapy**
Brexucabtagene autoleucel	▪ **Adults with R/R MCL** ▪ **Adults with R/R B-cell ALL**
Lisocabtagene maraleucel	▪ **Adults with large B-cell lymphoma** (including DLBCL NOS [including DLBCL arising from indolent lymphoma], high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B) that is: ▪ **Refractory to or relapsed within 12 mo of first-line chemoimmunotherapy** ▪ **R/R after first-line chemoimmunotherapy and not eligible for HSCT due to comorbidities or age** ▪ **R/R after ≥2 lines of systemic therapy**
Tisagenlecleucel	▪ **Adults with R/R large B-cell lymphoma after ≥2 lines of systemic therapy**, including DLBCL NOS, DLBCL arising from follicular lymphoma, high-grade B-cell lymphoma ▪ **Adults with R/R follicular lymphoma after ≥2 lines of systemic therapy** ▪ **Patients aged up to 25 yr with B-cell precursor ALL that is refractory or in second/later relapse**
BCMA-Targeted Therapies	
Idecabtagene vicleucel	▪ **Adults with R/R multiple myeloma after ≥4 prior lines of therapy**, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal Ab
Ciltacabtagene autoleucel	

CAR-T Mechanisms of Resistance

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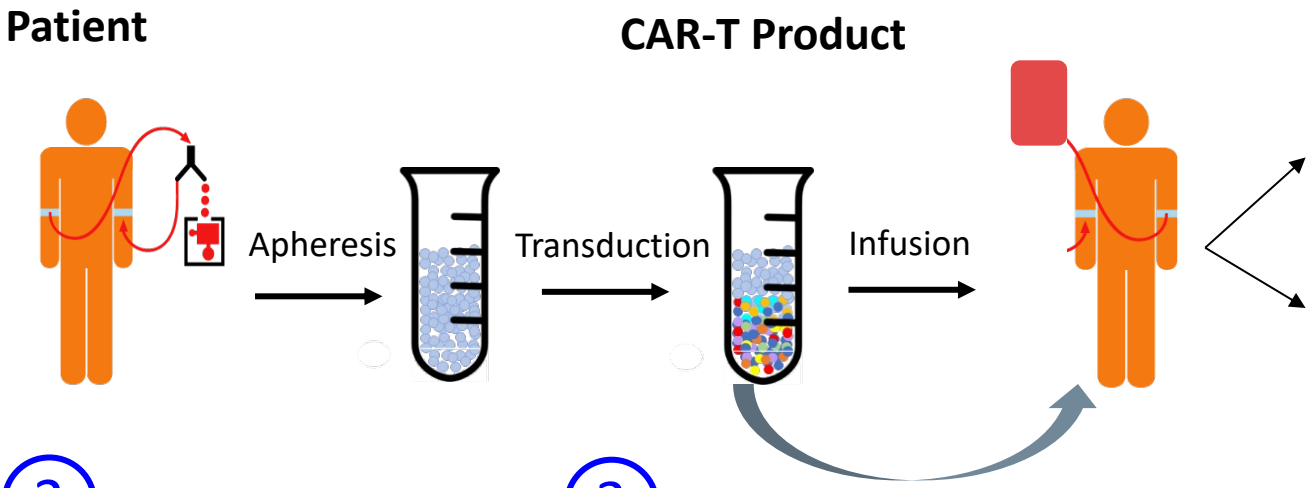
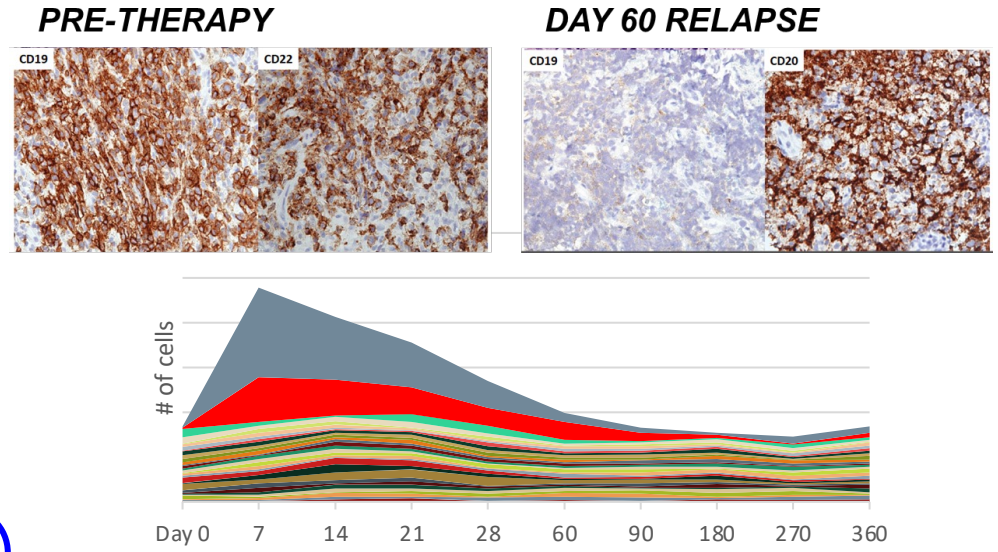


1

Tumor Biology:
CTL Tumor microenvironment predicts response
Tumor Antigen Density - **CD19 loss is a common MOF**
High Tumor Burden Predicts Poor Efficacy



2

T-Cell Fitness:
Prior Lines of Therapy
- Bendamustine
- Ibrutinib

T cell Effector cell origin
Allogeneic cell benefit?

3

CAR-T Manufacturing:
Virus transduction +/- gene edits
- IL2 or IL7 & IL15 plus CD28

Less expansion → avoid exhaustion
Minimize inhibiting Tregs
Patients need reliable production

4

CAR-T Pharmacokinetics and Pharmacodynamics:
Characterize which CAR-T localize to tumor & persist
- Immune phenotype and scRNA characterize CAR-T cells

Mechanisms of Relapse: Antigen Loss

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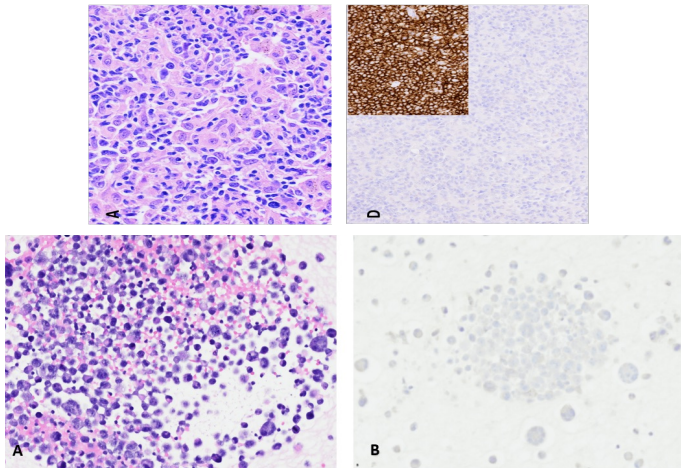
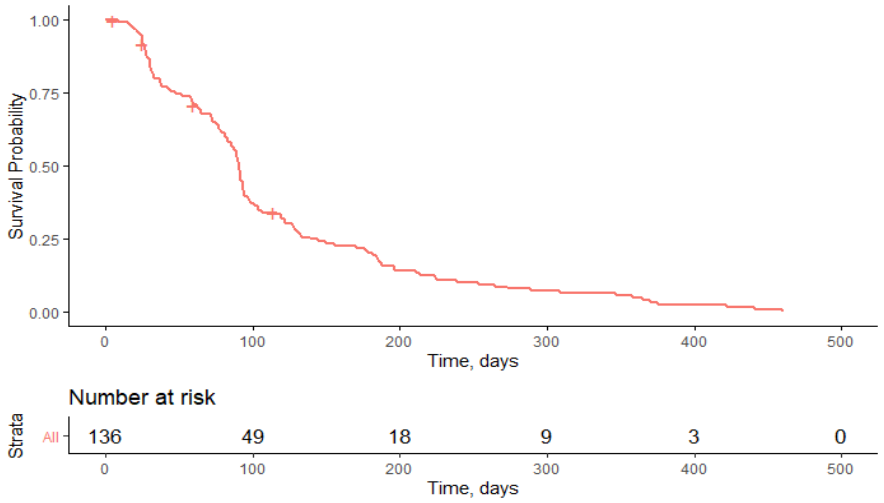
- Early on- several antigen negative relapses (intracellular/cytoplasmic CD19)

- US Lymphoma Consortium- **<u>30% (18 of 61) were CD19 negative.</u>**





mOS: 180 days
Median TTP: 91days (95% CI 83-93)

Bukhari...Dahiya. AJH. 2018 Speigel*, Dahiya*…Miklos. Blood. 2020

CAR22 Benefits Adults with rel/ref LBCL

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LBCL Key Eligibility Criteria

- R/R Lagre B cell Lymphoma
- **Prior CAR19 therapy, or CD19- disease**
- CD22 expression at any level

ASTCT Feb 2022
Matthew Frank, MD, PhD

If patient received prior CAR T-cell therapy

- > 30 days since CAR19 cell infusion
- < 5% circulating CAR19+ cells by flow cytometry

CAR22 Benefits Adults with rel/ref LBCL

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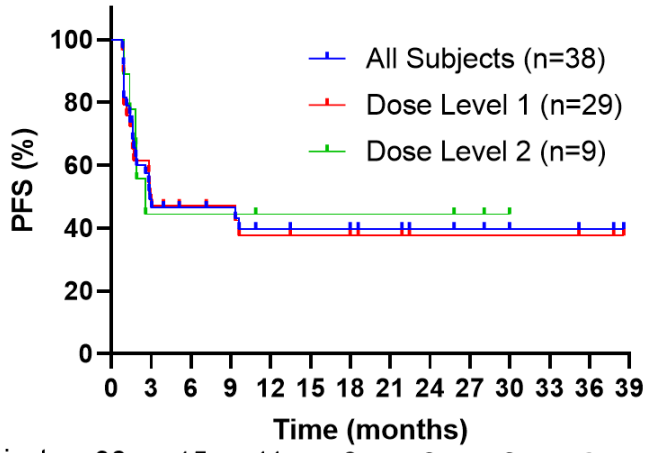
Summary:

- ORR is 68% Overall CR rate is 53%
- The ORR, CR rate, PFS and OS are similar between Dose Level 1 and 2
- CR are typically durable
  - Only 2 of the 20 patients who achieved a CR has relapsed
- Dose Level 1 is the recommended Phase 2 dose.
  - 1 million CAR22+ cells/kg

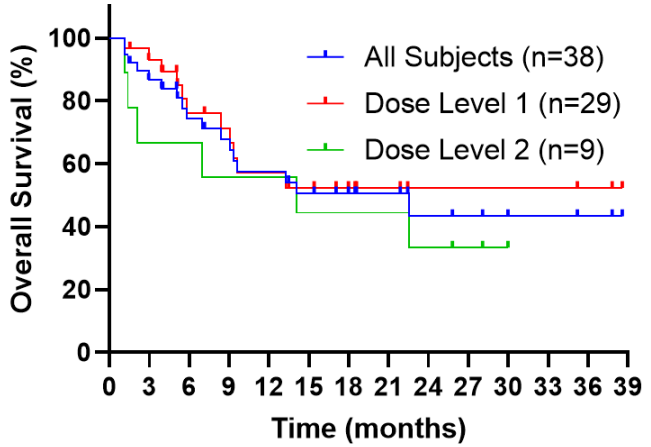
LBCL	DL1 (N = 29)	DL2 (N = 9)	Tot (N = 38)
Median follow up, months [range]	14.1 [1.5-38.6]	27.1 [24.7-33.5]	18.4 [1.5- 38.6]
Overall Response Rate (ORR)*, n (%)	19 (66%)	7 (78%)	26 (68%)
CR Rate	15 (52%)	5 (56%)	20 (53%)
Median PFS (months, 95% CI)	3.0 (1.6 -NR)	2.6 (1.3 - NR)	2.9 (1.7 - NR)
Median Survival (months, 95% CI)	NR (8.3 - NR)	22.5 (5.5 - NR)	22.5 (8.3 - NR)

Progression Free Survival



All Subjects:	38	15	11	9	6	3	2
Dose Level 1:	29	11	8	6	3	3	2
Dose Level 2:	9	4	3	3	3	0	0

Overall Survival



All Subjects:	38	23	17	11	6	3	2
Dose Level 1:	29	17	12	7	3	3	2
Dose Level 2:	9	6	5	4	3	0	0

*Response was investigator-assessed using Lugano criteria for LBCL.

Simultaneous targeting of two tumor antigens may overcome antigen loss and improve efficacy

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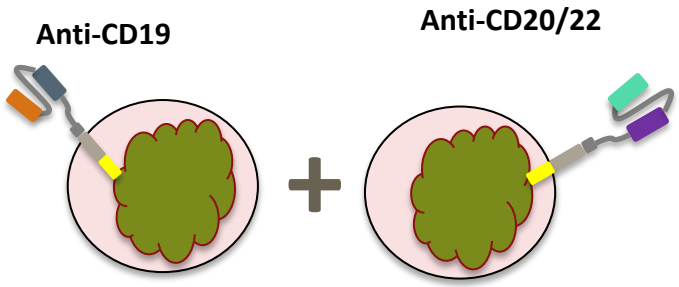
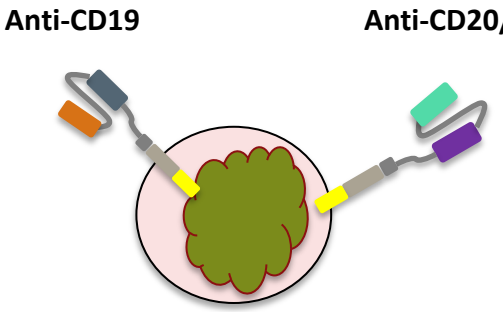
Co-administration



Pros:
- Defined dose for each CAR

Cons:
- Multiple production runs
- Potential competition
- When to infuse 2nd dose

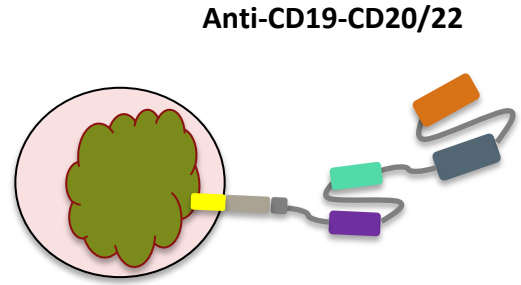
Co-expression
(co-transfection or bicistronic)



Pros:
- Each CAR molecule signals independently
- Reduces steric concerns

Cons:
- Can generate multiple CAR populations

Bivalent-bispecific receptor



Pros:
- Each cell expresses both scFVs

Cons:
- Distal scFV may have signalling deficiencies

Cronk RJ, *et al. Cancers* 2020; 12:2523.

CAR-T Targeting both CD19 and CD20 Simultaneously

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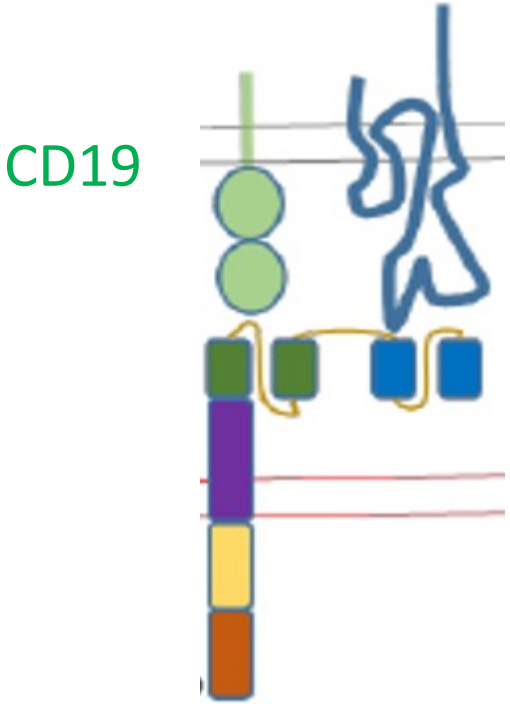
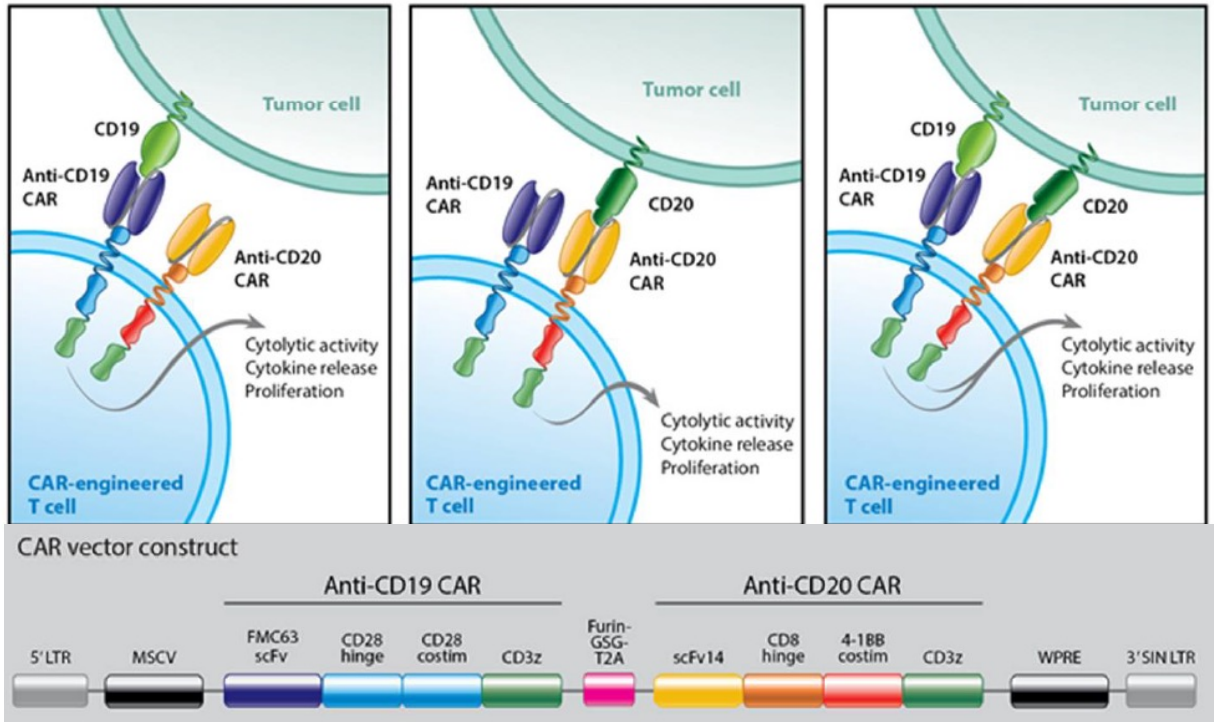
Miltenyi MB-CART2019.1
Single polypeptide bispecific anti-CD19 and CD20 CAR Therapy



Kite 363: Bi-cistronic co-Expression of anti-CD19 and CD20 CAR Therapy



Pro: Anticipate Axi-Cel efficacy without progression due to antigen loss

Con: Potentially more toxicity

Nirav Shah et al. Nature Medicine 2020; NCT04792489

New Unmet Medical Need :
2L CAR-T for Rel/Ref LBCL with Elevated LDH

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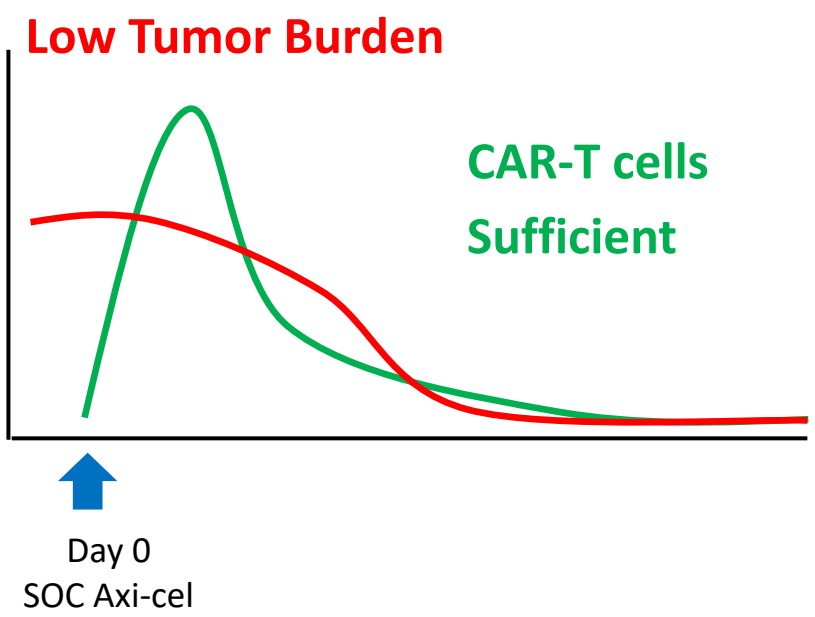
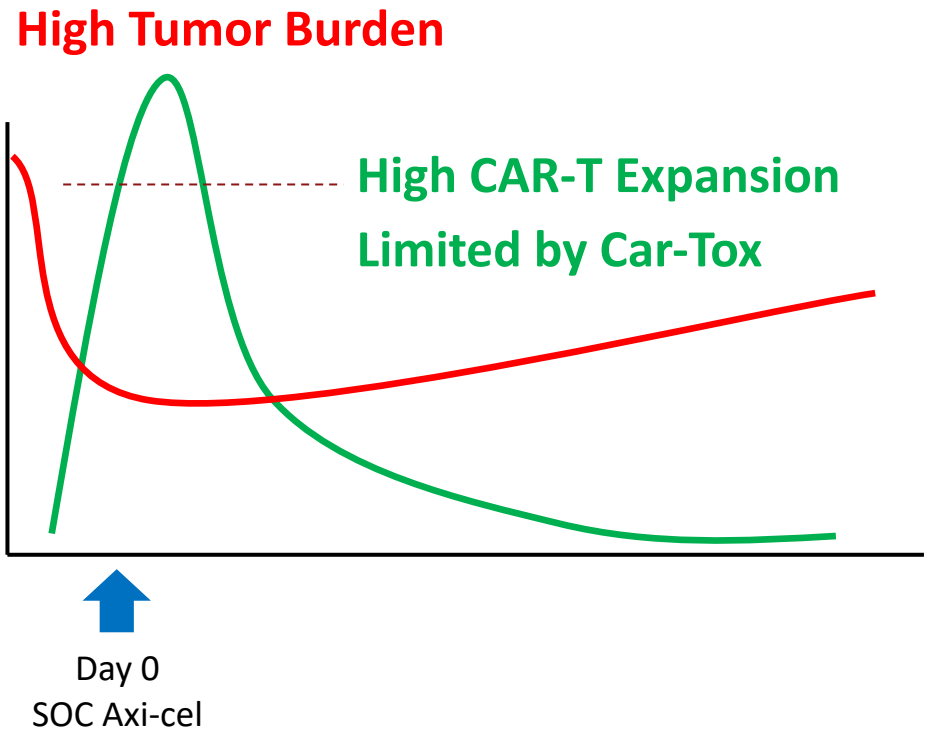
Normal LDH



Elevated LDH



Dose Escalation using Second Axi-Cel Infusion in Patients with High Tumor Burden (Axi-Cel x 2)

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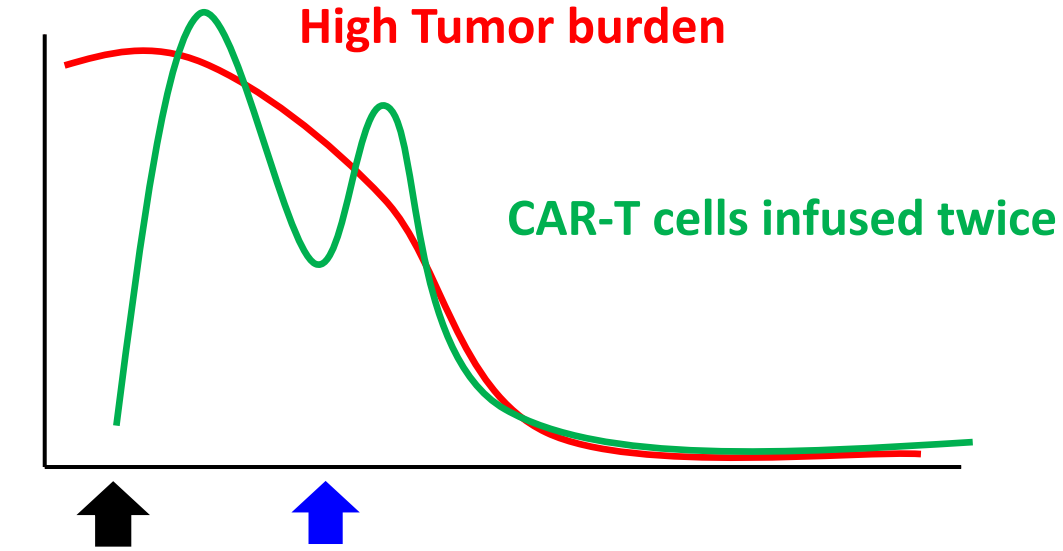
Elevated LDH

Reinfuse 2nd dose of Axi-cel between days 7-14



Hypothesis:
2nd dose of Axi-cel within 7-14 days improves efficacy for Lymphoma patients with high tumor burden

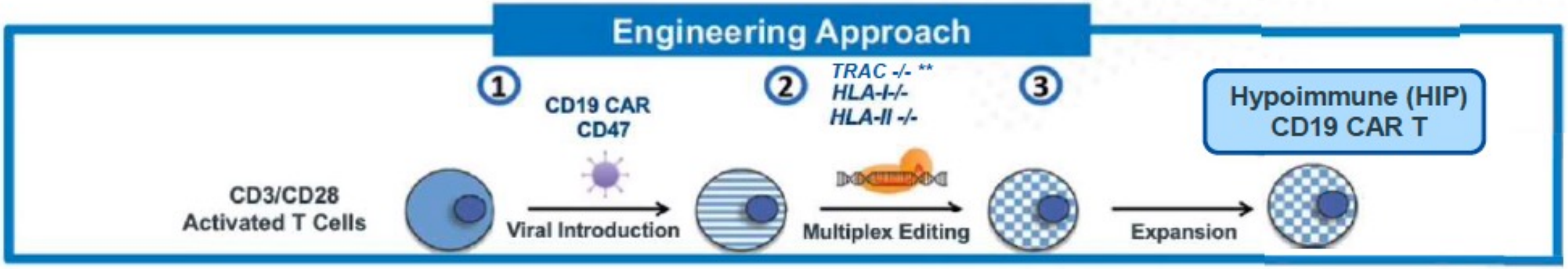
1. **Improved Efficacy**
   2nd product expansion improves effector to target ratio
2. **LD conditioning persists thru day 14**
3. **Second infusion may overcome CAR-T exhaustion**
   - Zuma 1 correlative studies showed relapse associating with 3 exhaustion markers on day 7 (Locke. Blood Adv 2020)
   - **AxiCel x2** provides non-exhausted CAR-T
4. **Minimize CAR-T toxicity**
   - Two product strategy allows for conservative toxicity management using EARLY steroids for SOC Axi-Cel CAR-TOX
   - Tumor debulking by 1st SOC Axi-Cel enables *fresh* **Axi-Cel x 2 to eradicate remaining LOW burden tumor**

A Growing Number of CAR Targets Are Being Studied

Tumor Type	Targets Currently Being Investigated
Acute lymphoblastic leukemia (ALL)	CD5, CD7, CD19, CD22, ROR1, BCMA, glypican-3 (GPC3), CLD18, CLL-1, BAFFR
Acute myeloid leukemia (AML)	CD33, CD34, CD38, CD56, CD117, CD123, CD133, LEY, MUC1, FLT3,
Astrocytoma	HER2, EGFRvIII, IL13Rα2
Breast	HER2, EpCAM, cMET, mesothelin, ROR1, MUC1, CEA, CD70, CD133
Chronic lymphocytic leukemia (CLL)	ROR1, Igκ, CD19, CD20
Chronic Myelogenous leukemia (CML)	IL-1RAP
Colorectal	CEA, EGFR-IL12, MUC1, HER2, NKG2D
Fallopian	MUC16
Glioblastoma	HER2, EGFRvIII, IL13Rα2, EphA2
HCC	GPC3, MUC1, EPCAM, c-Met/PD-L1, BCMA, CD19, CLD18, CD147

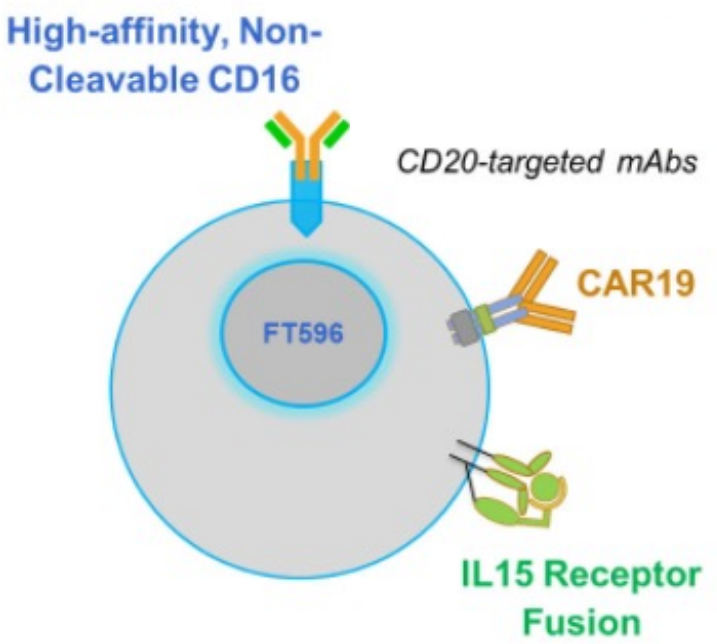
Tumor Type	Targets Currently Being Investigated
Lymphoma	CD4, CD5, CD7, CD19, CD20, CD22, CD30, CD33, CD37, GPC3, BCMA, CD19, CLD18, ROR1
Melanoma	cMET, GD2, CD20, CD70, VEGFR2
Mesothelioma	Mesothelin
Multiple myeloma (MM)	BCMA, CD19, CD138, CD56, CD38, CS1, NY-ESO-1, LeY, Igκ, GPC3, CLD18, CD269, GPRC5D, SLAMF7
Neuroblastoma	GD2, CD171
NSCLC	PD-L1, MUC1, ROR1, NY-ESO
Ovarian	Mesothelin, CD70, HER2, CD133, FAP, nectin-4, MUC16
Pancreatic	Mesothelin, prostate stem cell antigen (PSCA), CD70, MUC1, HER2, CEA, BCMA, GPC3, CD19, CLD18
Peritoneal	MUC16
Prostate	Prostate-specific membrane antigen (PSMA)
Stomach	EPCAM, CEA, MUC1, HER2, CLD18
Thyroid	ICAM-1

ALLO-CARs

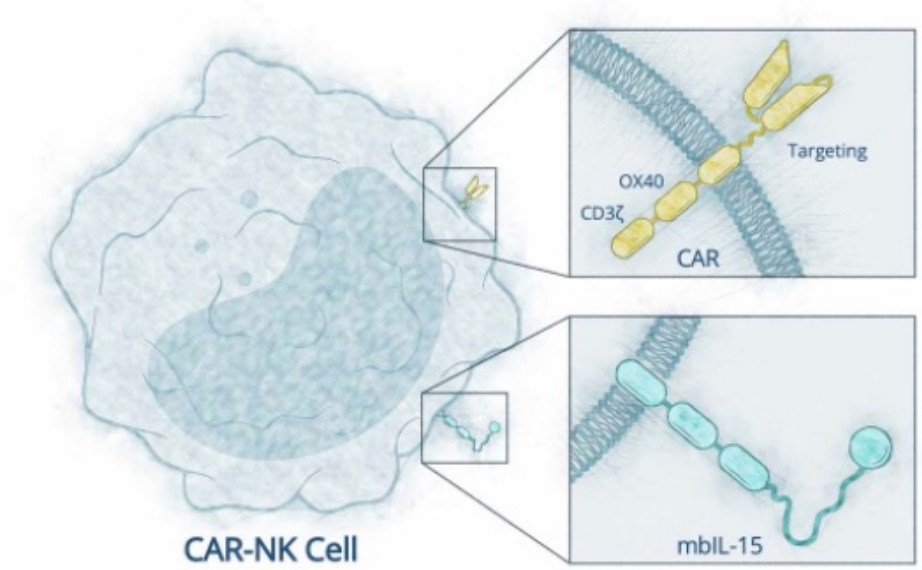


** T-cell receptor (TRAC) gene is also deleted to prevent GvHD

NK cell engineering



- *Potent CAR tailor-made for NK cell anti-tumor activity*
- *hnCD16 to universally engage mAbs and mitigate antigen escape*
- *IL15/R to enable NK cell persistence without the need for cytokine support*



Targeting receptor, OX40 costimulatory domain, CD3ζ signaling moiety, membrane bound IL-15

Challenges in Cell Therapy in Solid Tumor

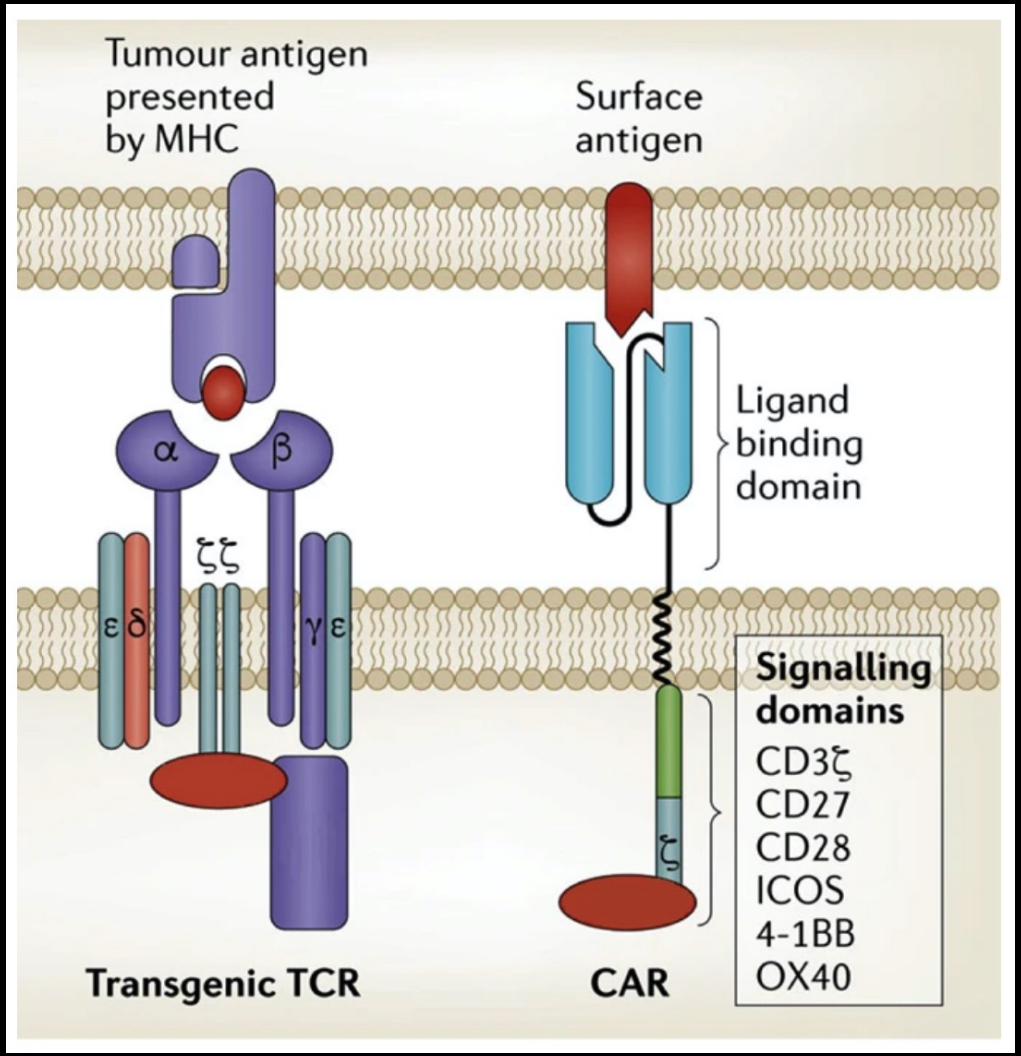
Target(s)

- Most of the targeted antigens are intracellular molecules, requiring MHC presentation
- Targetable surface protein(s) are usually shared by normal cells

Delivery

- Solid tumor sites can be difficult to reach by the immune cells
- The tumor micro-environment can be hostile

MHC-restricted Ag

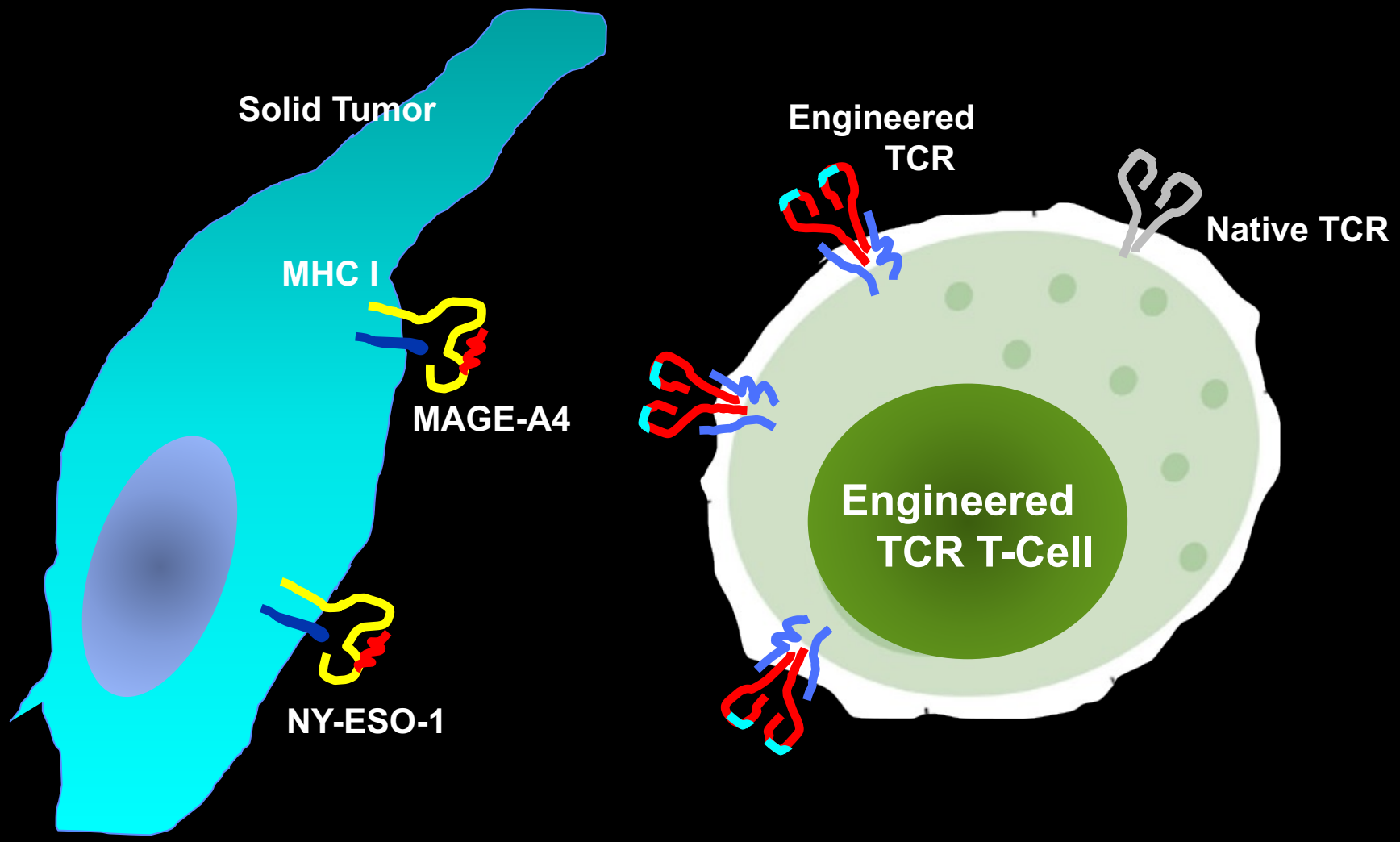


Surface Ag

Engineered TCR T-cell
Need a known TCR
Native machinery

CAR T-cell
Technically complex
Artificial machinery

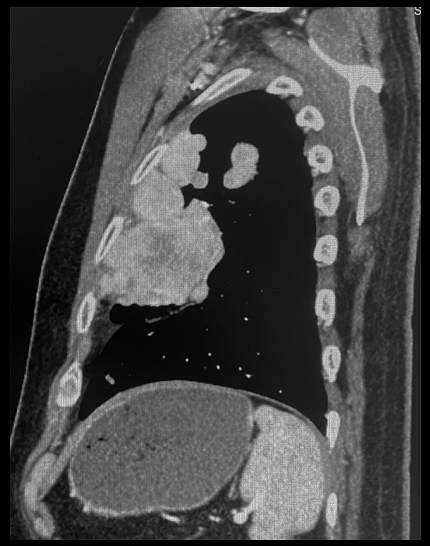
Engineered TCR T-Cells



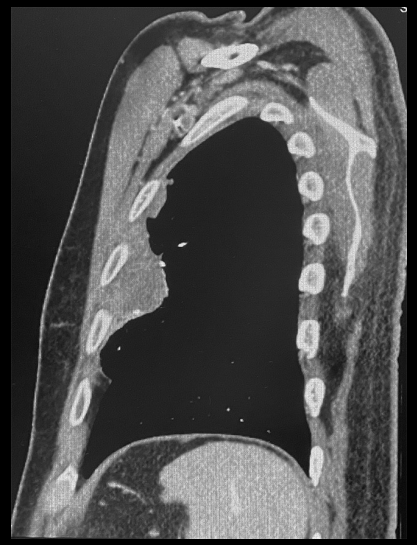
Engineered Autologous TCR T-Cells

Synovial Sarcoma

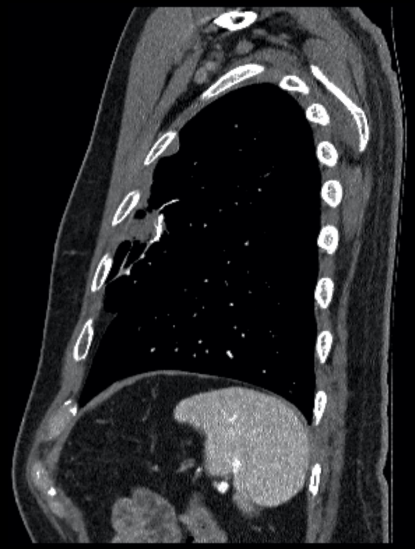
Pre



Day+28



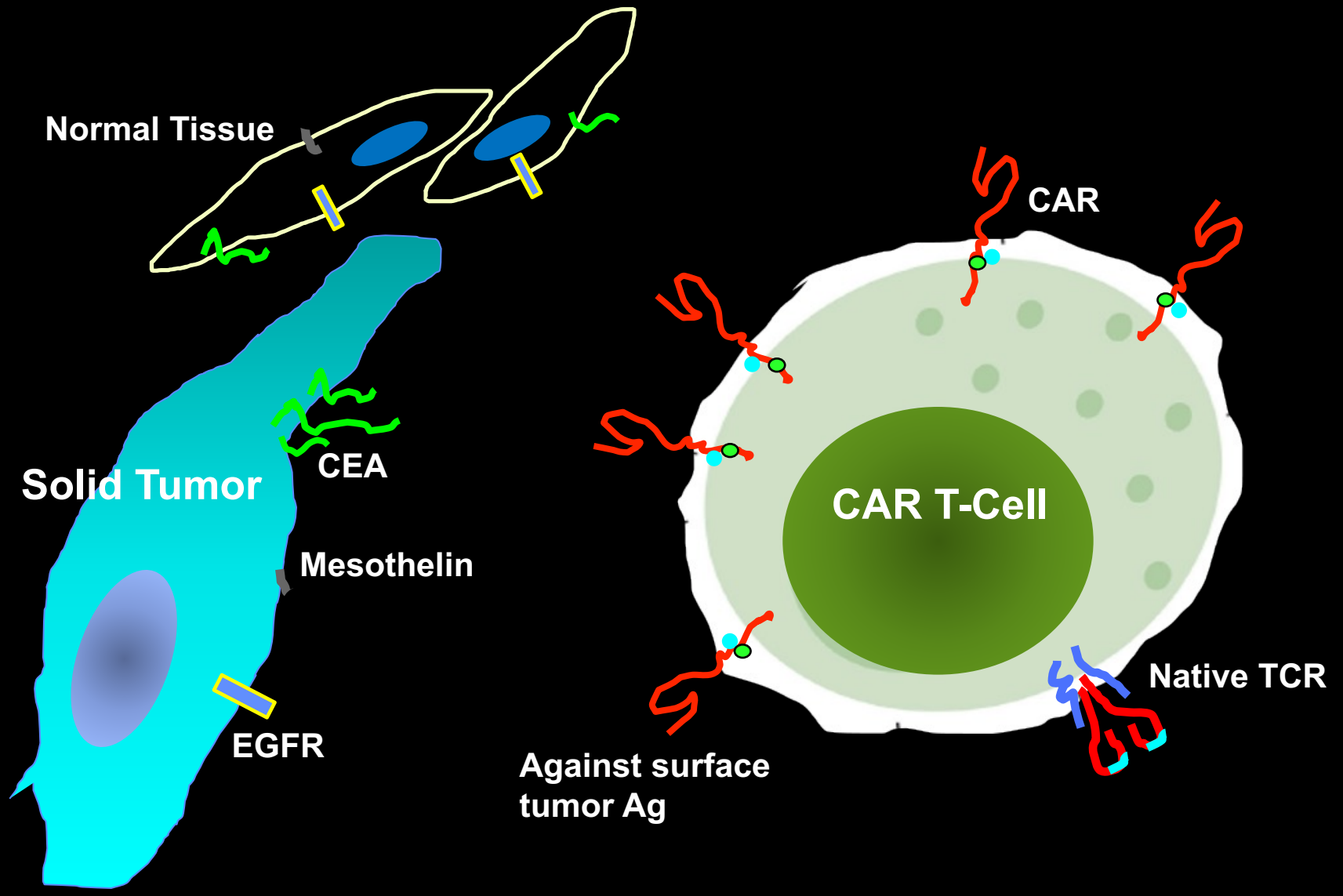
Day+90



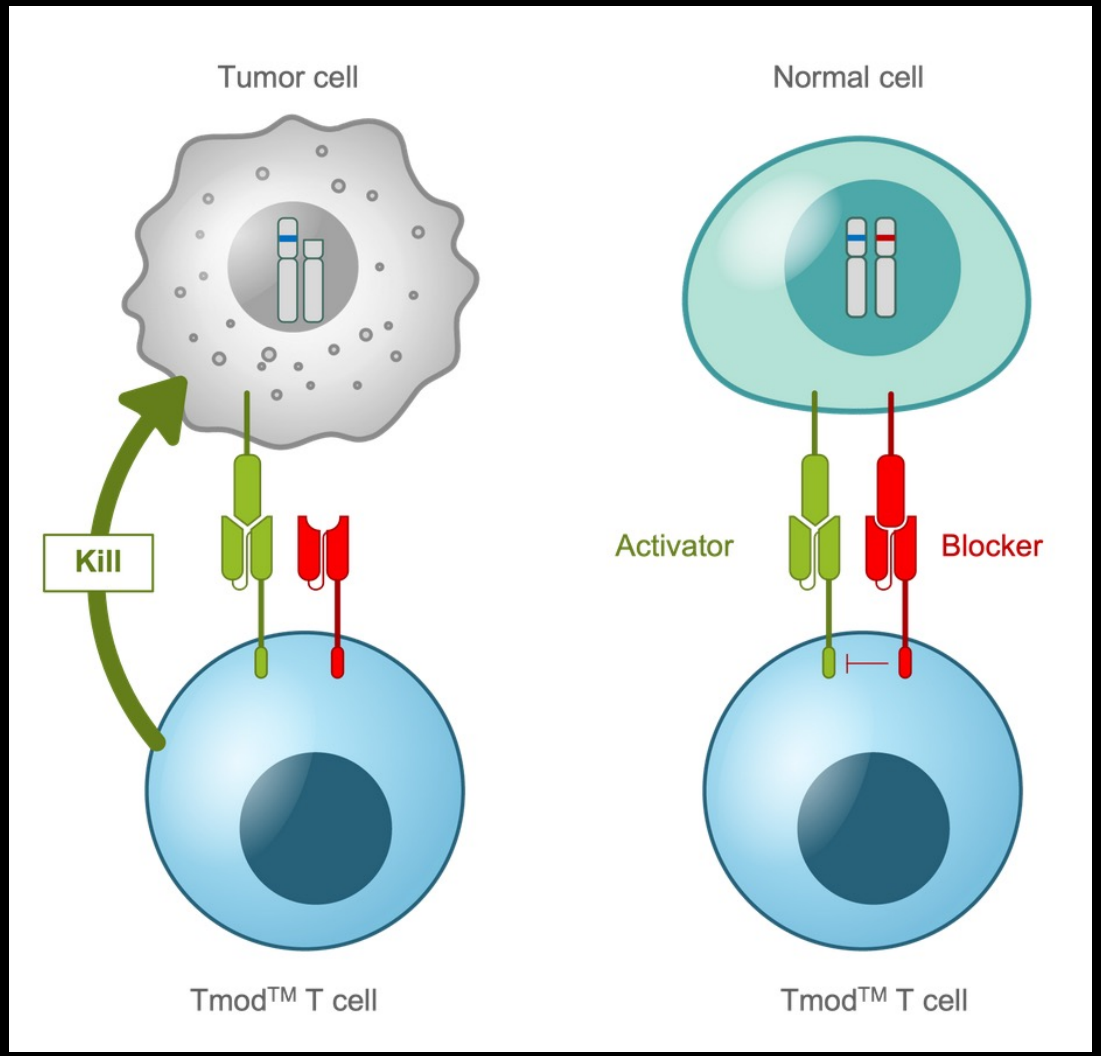
Engineered TCR against MAGE-A4 in HLA-A2 patients

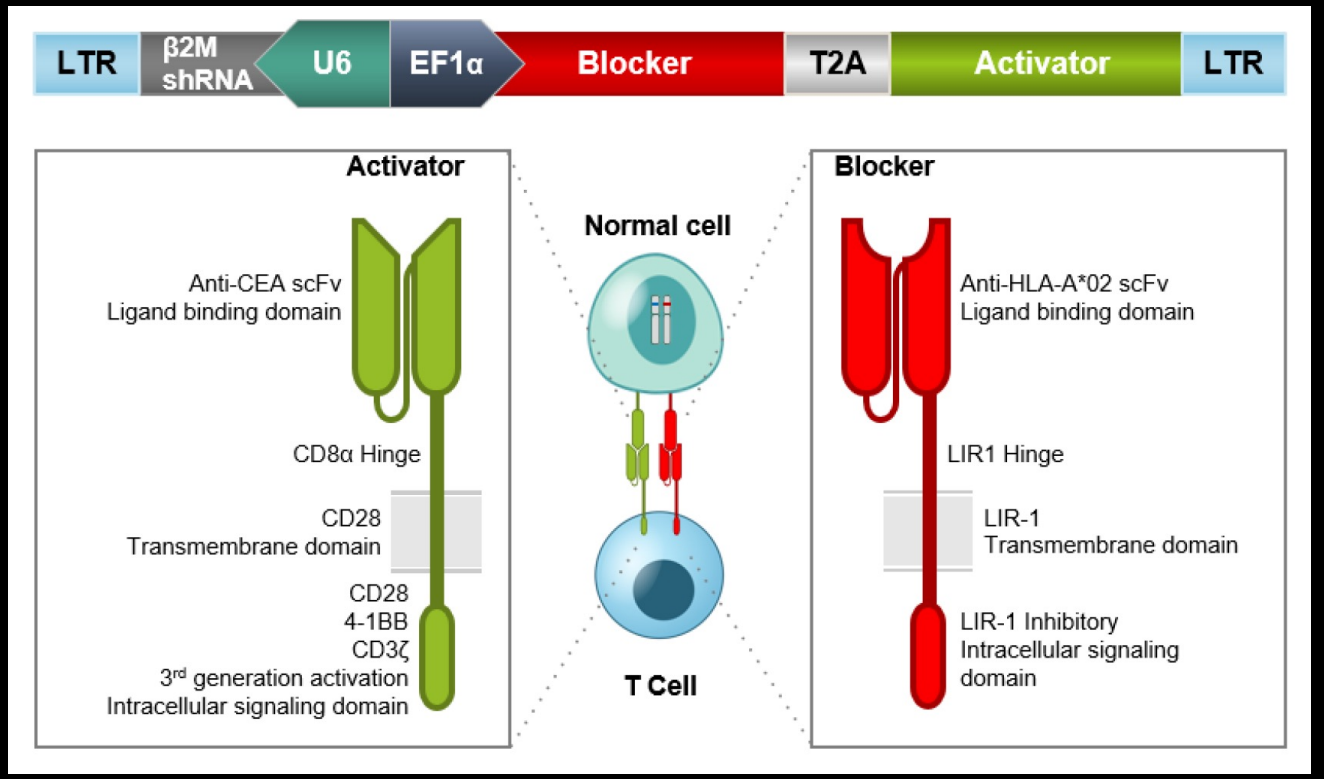
Main and Disease PI: Kristen Ganjoo (Oncology)
CCT PI: Wen-Kai Weng
Sponsor:

Potential Surface Target(s) for CAR T-Cells in Solid Tumors



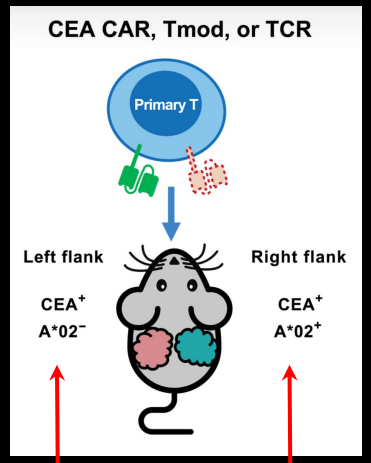
Double CAR T-Cells with a Blocker (Tmod)



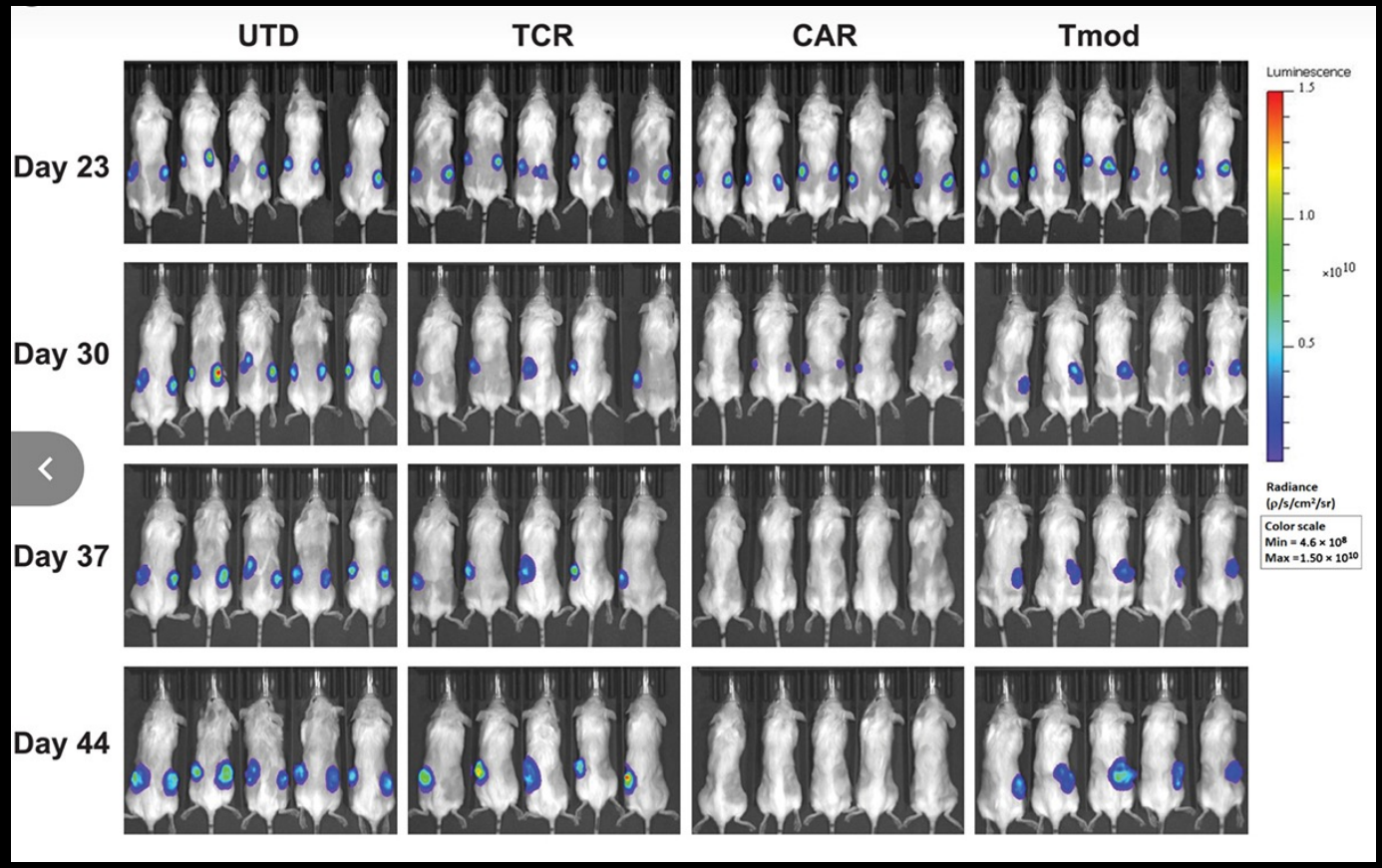


Sandberg et al. Science Translational 2022, 14:

Tmod CAR T-Cell Killing is Very Specific



UTD	No treatment
TCR	with TCR recognizing CEA within HLA-A2
CAR	Anti-CEA CAR
Tmod	Anti-CEA CAR with HLA-A2 blocker

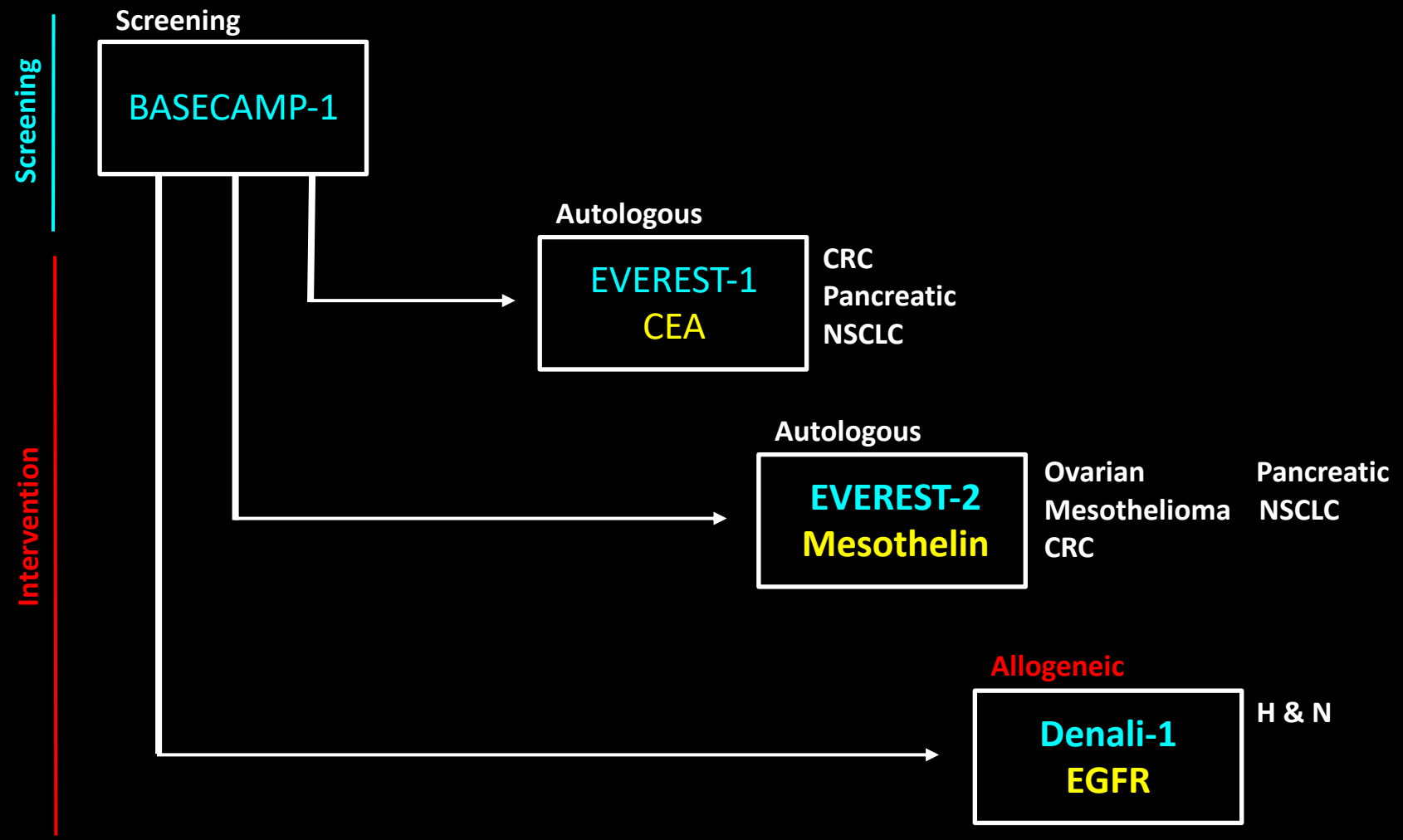


Frequency of LOH in HLA-A in Common Cancers

Tumor Type	TCGA Frequency of HLA-A LOH
Glioblastoma	0.1456
Breast	0.1361
Cervix	0.1424
Colorectal	0.0959
Esophagus	0.2337
Kidney & renal	0.1875
Liver	0.0243
Lung squamous cell carcinoma	0.2535
Lung adenocarcinoma	0.1163
Melanoma	0.0650
Head and neck	0.1609
Ovary	0.1710
Pancreas	0.3315
Prostate	0.0447
Stomach	0.1315
Thyroid	0.0080
Urinary bladder	0.1838
Uterine	0.0315

The Cancer Genome Atlas (TCGA)

The Strategy Using Tmod for Solid Tumor



Screening Study : BASECAMP-1

Non-interventional

- Screen patients for the 3 interventional study
- Target patients with HLA-A0201 heterozygous and whose tumors of LOH in HLA-A0201

Tasks

- Genetic testing of patients and their tumors
- Collect autologous PBMC via apheresis in eligible patients

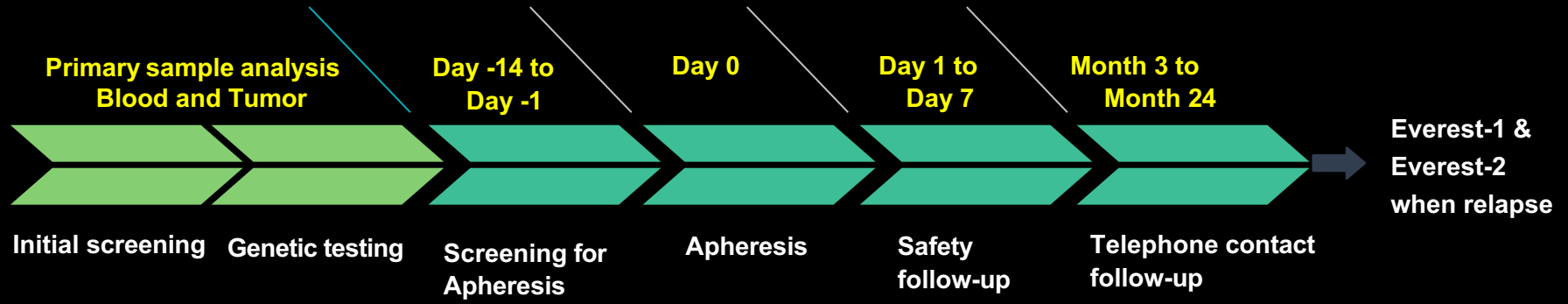
Screening Study : BASECAMP-1

Screening Part 1: Labcorp HLA haplotype (blood or buccal)

- To identify HLA-A*02:01 heterozygous patients

Screening Part 2: Tissue block sent to Tempus and possible apheresis

- Require >40% tumor purity
- FFPE quality for NGS library generation
- Results include molecular data on 648 genes + HLA LOH
- Apheresis and product freezing of eligible patients



EVEREST-1：CEA-Targeting Tmod CAR T-Cells

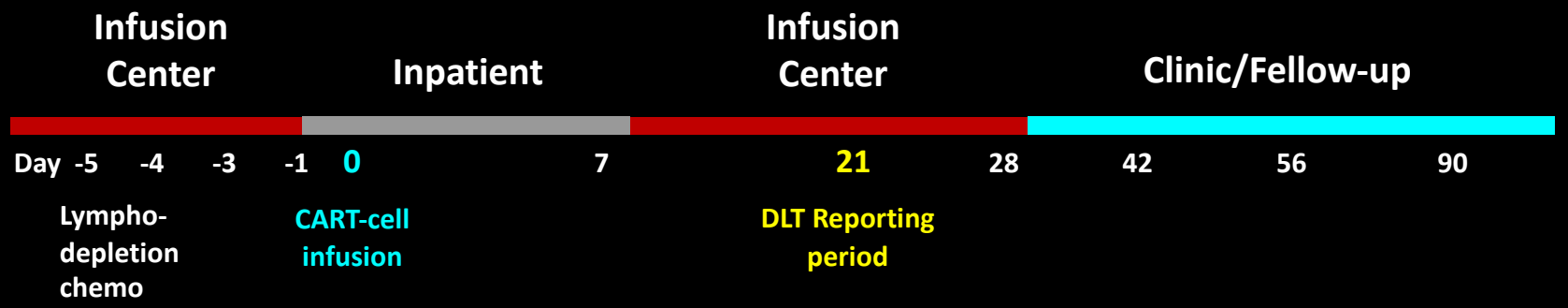
Tumor types and Candidates

- Colorectal, Non-Small Cell Lung, Pancreatic
- Patients with high risk of relapse
- Treating upon relapse

CAR T-cell therapy

- A2B530: Anti-CEA activating CAR (CD28) with Anti-HLA-A2 blocker
- Standard LD chemotherapy

EVEREST-1 : CEA-Targeting Tmod CAR T-Cells



- Lympho-depletion (LD) chemo (day -5, -4, -3)
  - Fludarabine 30 mg/m^2 x3 days
  - Cyclophosphamide 500 mg/m^2 x3 days
- Admission on day -1 and plan to discharge on day +7
- Infusion center (ITA) monitoring until day +28
- Patients stay within 2 hours driving distance until day +42

CAR T Therapy: Future Directions

- Overcoming mechanisms of resistance
  - T-cell exhaustion: combination therapy with checkpoint and other immunomodulatory agents, gene editing out immunomodulatory genes (ie, PD-1); (Staudtmeuer et al, NY-ESO-1 TCR-T, PD-1KO)
  - Antigen loss: multi-antigen targeting CARs (CD19/CD20; CD19/CD22, CD19/CD79b, CD19/20/22)
  - Improve functionality

- Composition of the T-cell product (shift towards an early memory differentiation phenotype)
  - Pre-leukapheresis and/or conditioning regimens (BTK inhibitors, PI3K inhibitors); postleukaphersis T-cell/product manipulation (PI3K inhibitors)
- Increasing accessibility (cost and manufacturing time being rate limiting factors)
  - Allogeneic CAR T-cells, CAR-NK, other cells
  - POC production

Summary

- New frontier- Cellular Immunotherapy.
- Unprecedented response rates seen with the use of CD 19 CAR-T therapy in rel/ref DLBCL, myeloma and ALL.
- Challenges- immediate and long-term side effects (CRS, Neurotoxicity), and cost.
- ‘Halo Effect’ for clinical research.
- Early referrals will ensure that both efficacy and safety are optimized, as outcomes are associated with patient fitness, T-cell fitness, and disease burden.
- Earlier and more aggressive CRS and NT mitigation strategies have decreased high-grade toxicities, allowing for treatment of a broader patient population.
- Emphasis on **collaboration, collegiality, alignment, and compassion** in delivering these complex therapies (CAR-T therapy, BMT).