ELLIS FISCHEL CANCER CENTER

CAR T cell therapy: Past, present, and future

Gerhard Hildebrandt, MD, FACP

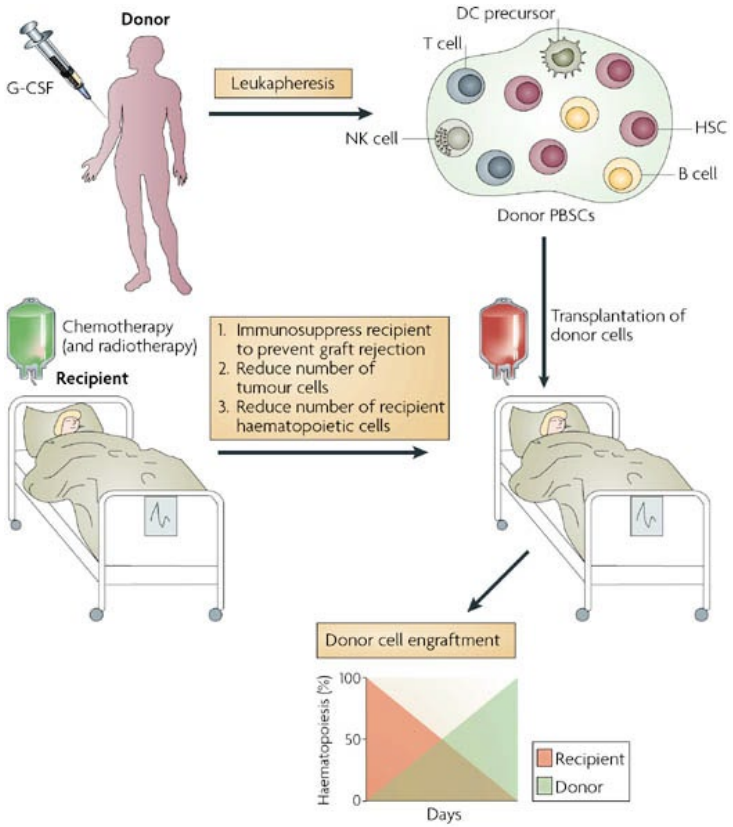
Professor of Medicine

Gerhard.Hildebrandt@health.missouri.edu

MU Health Care

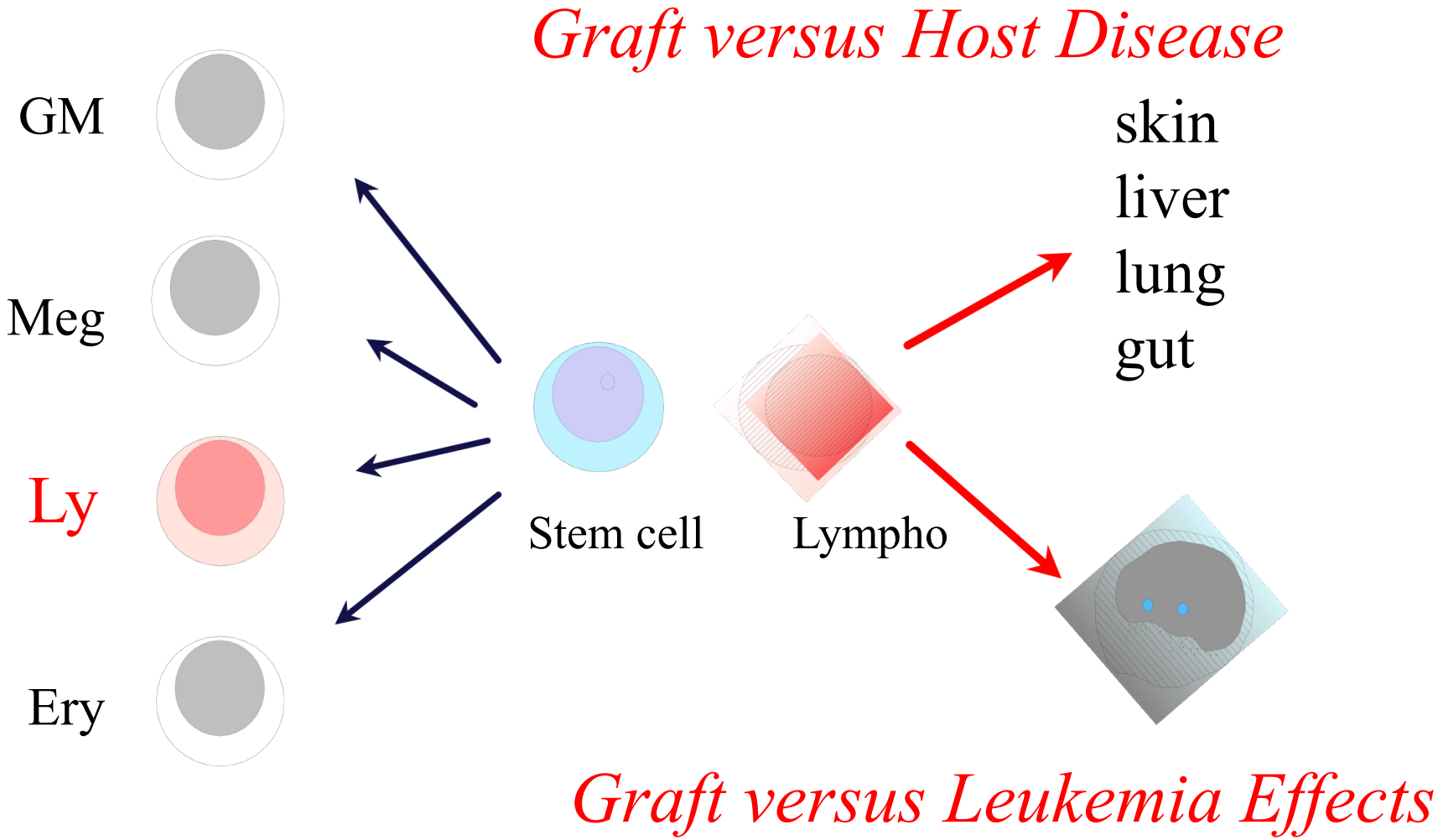
Allogeneic Hematopoietic Cell Transplantation

- First established cellular therapy
- Utilizing healthy immune cells from a donor to fight cancer in the patient
- Only curative option for several hematologic malignancies such as acute leukemia
- Historically the only curative option for refractory lymphoma and myeloma



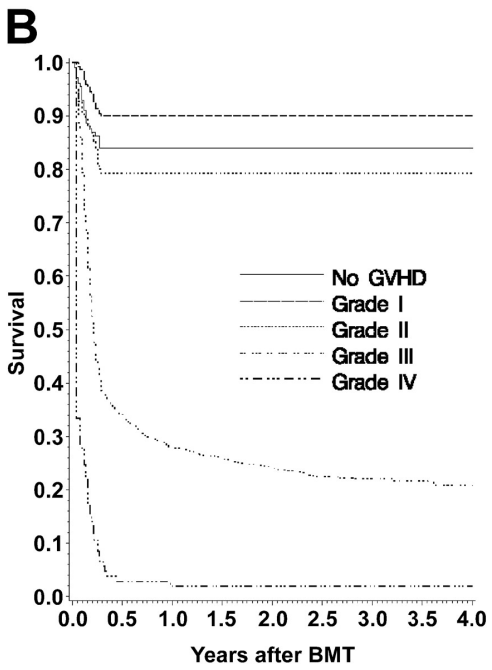
Warren D. Shlomchik
Nature Reviews Immunology **7**, 340-352 (May 2007)

The T cell dilemma of allogeneic HCT



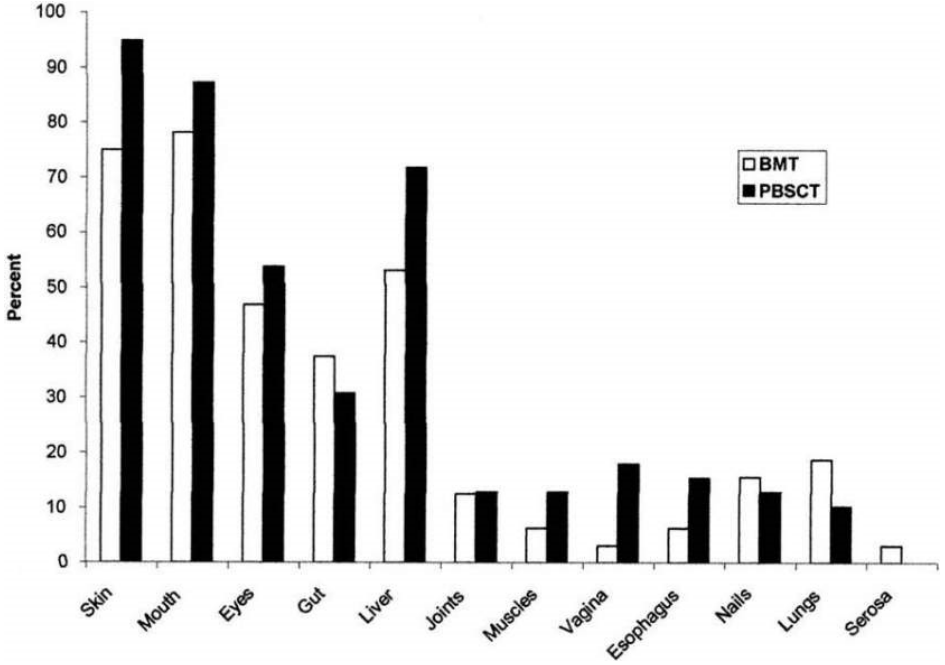
Significant complicatios and mortality risk with allo HCT

Acute GVHD severity and survival



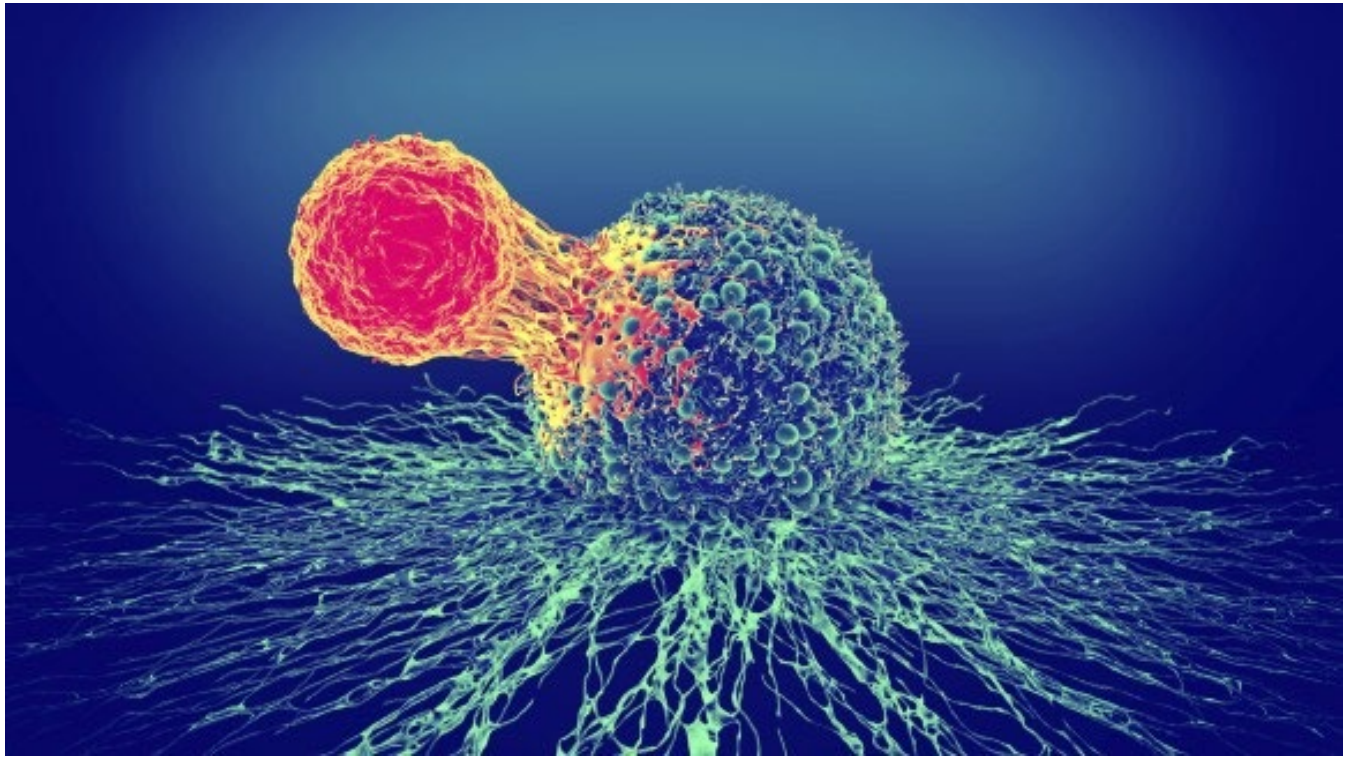
Cahn, J.-Y. et al. Blood 2005;106:1495-1500

Chronic GVHD



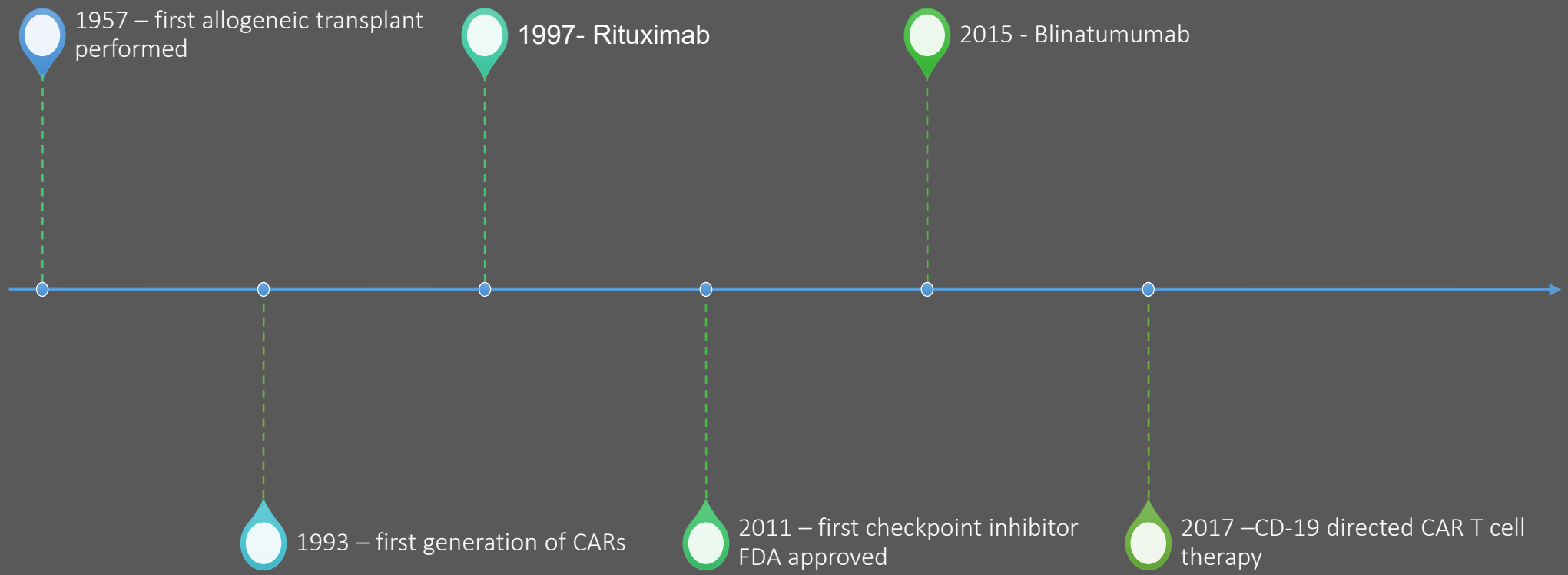
Flowers et al. Blood 2002;100:415-9

Chimeric antigen receptor – T cell (CAR-T cell): Today's and tomorrow's missile to eradicate cancer

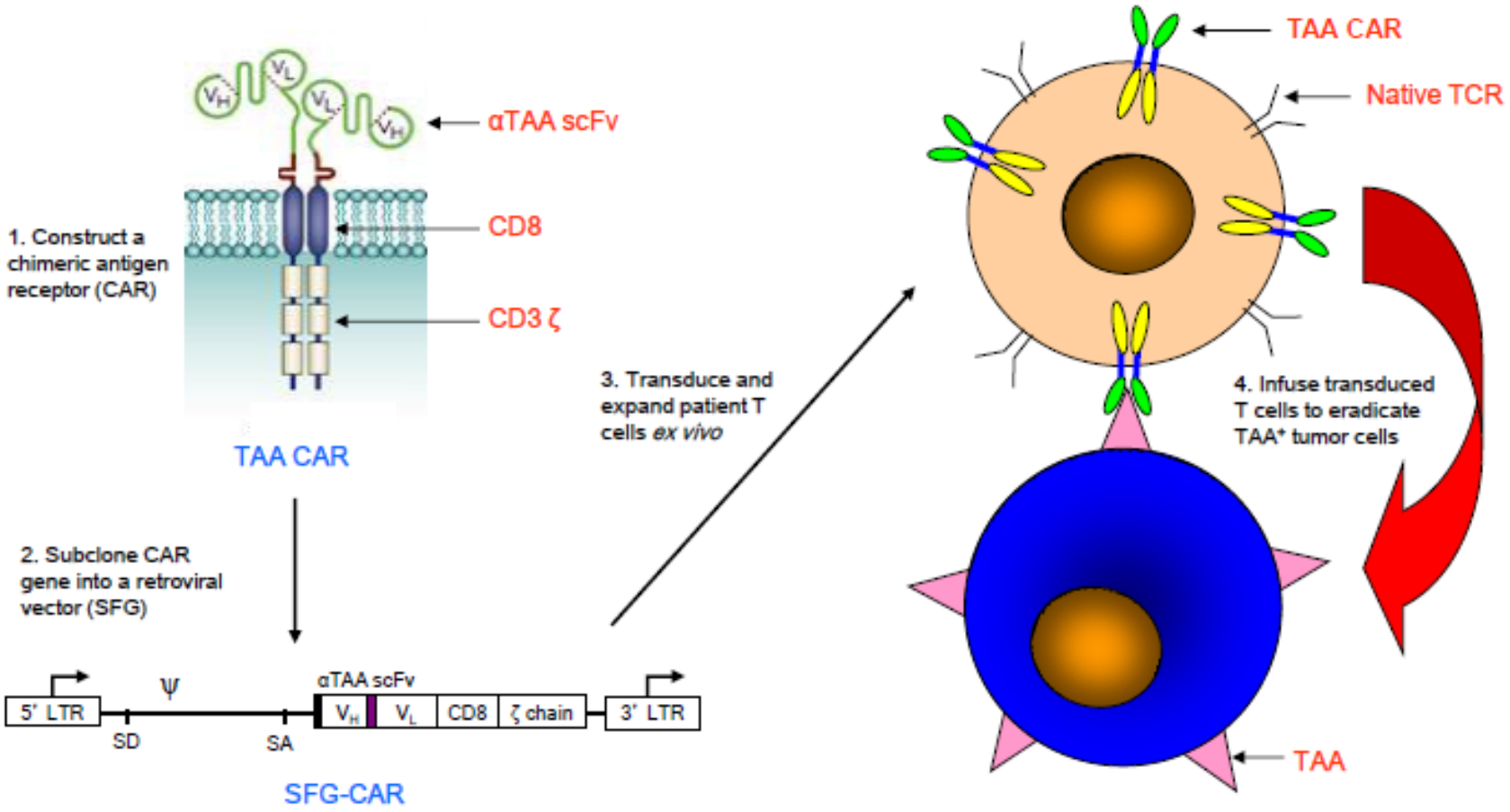


https://www.nature.com/collections/dcbdhfibgi

Cancer and Immunity: Timeline of Progress

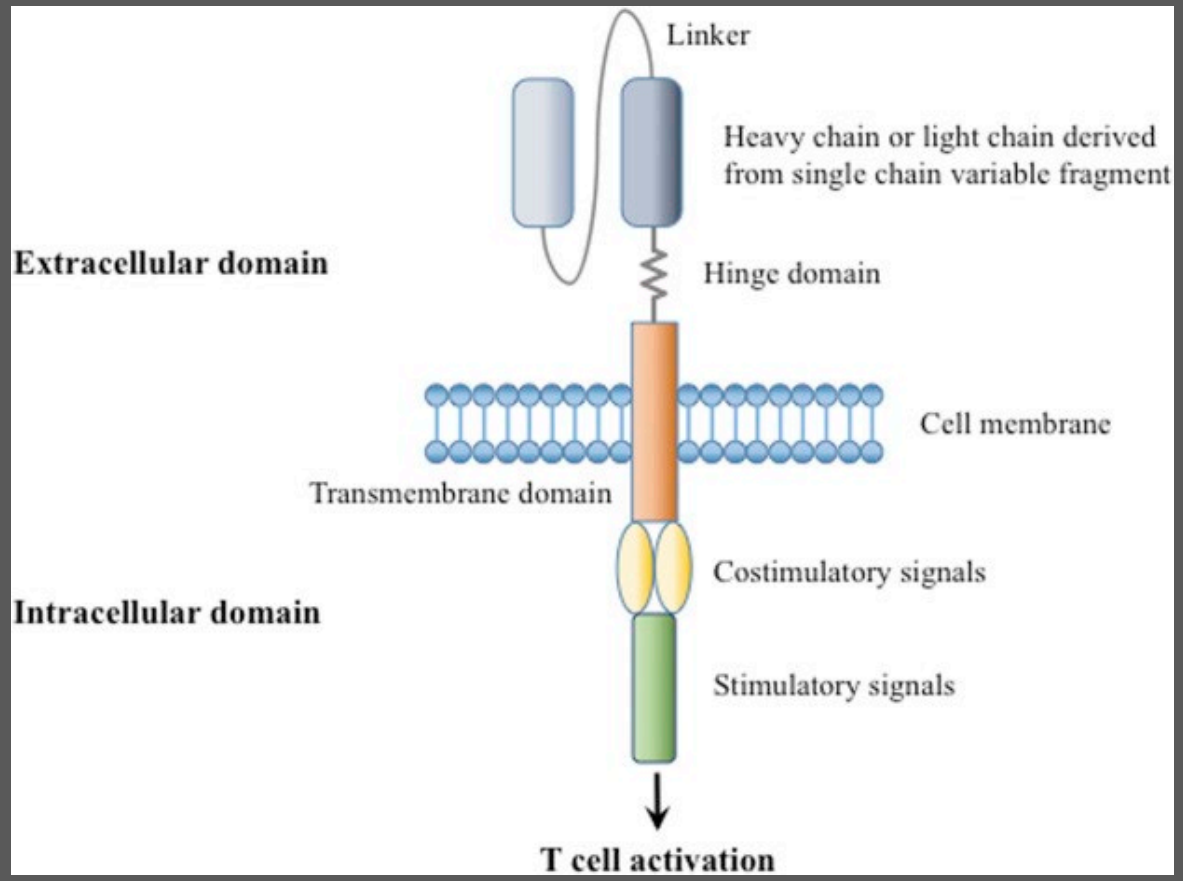


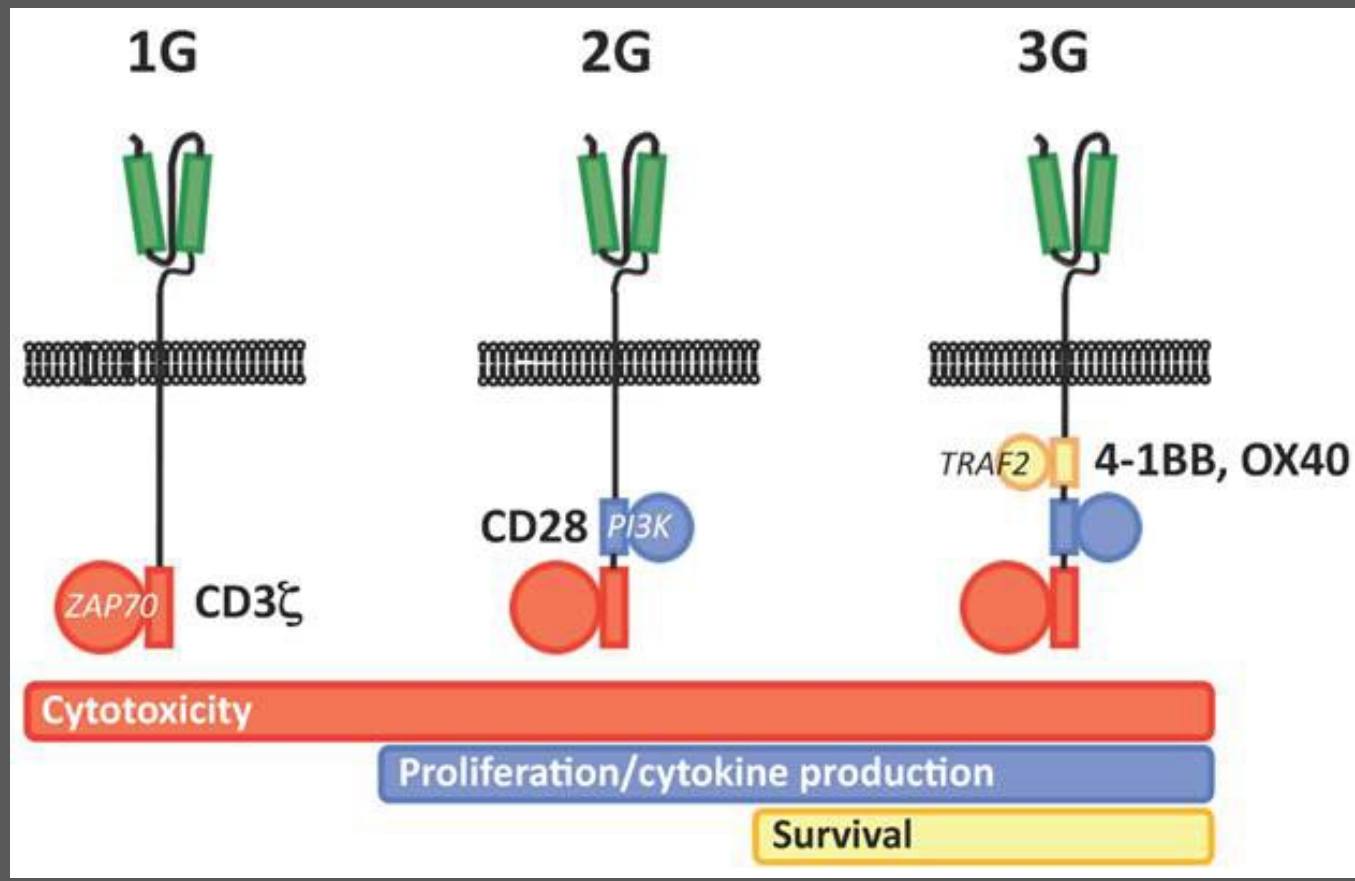
CAR and Creation of CAR T-Cells



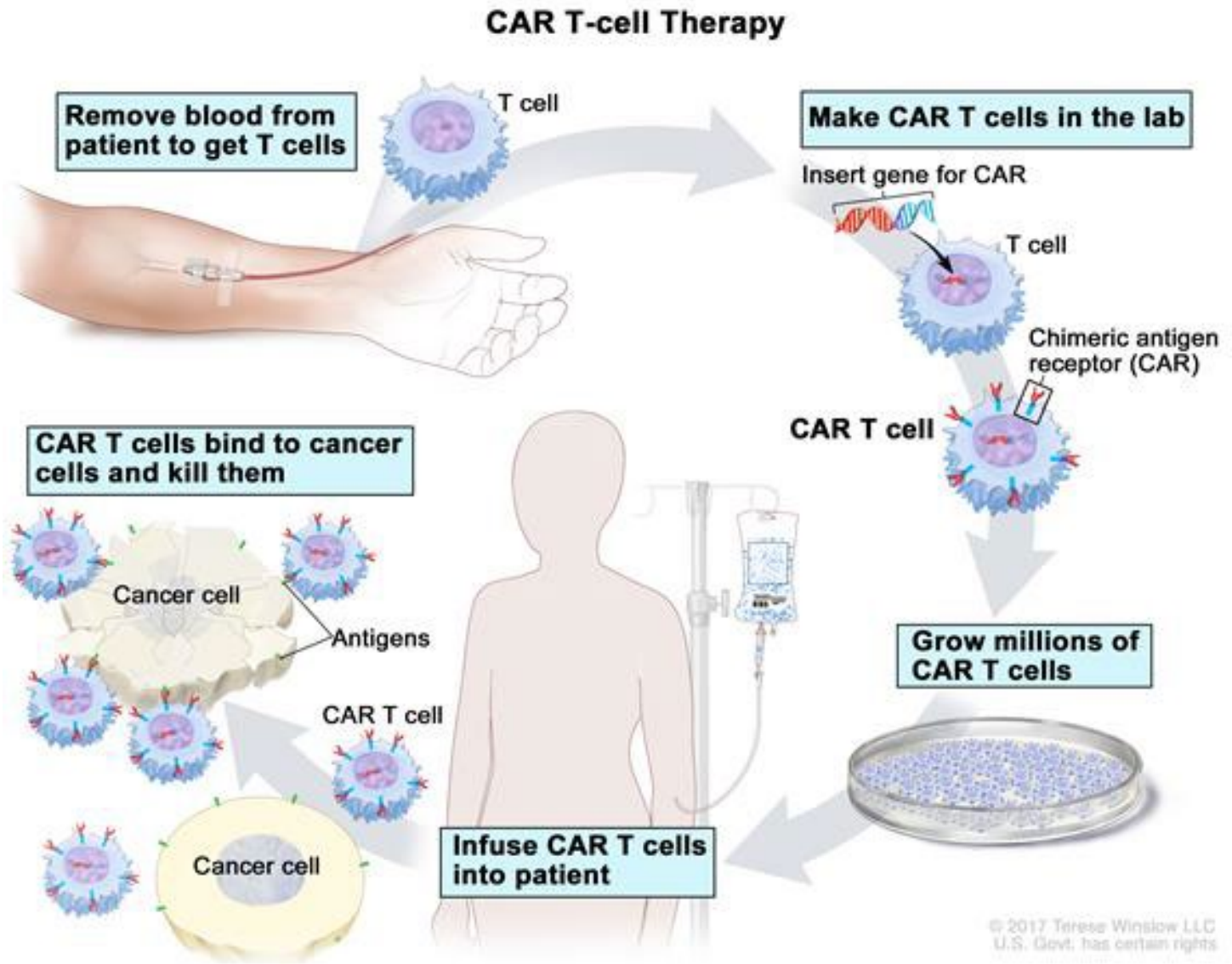
Courtesy of R. Brentjens

CAR and Creation of CAR T-Cells





Making CAR T-Cells



FDA Approved CAR T Products

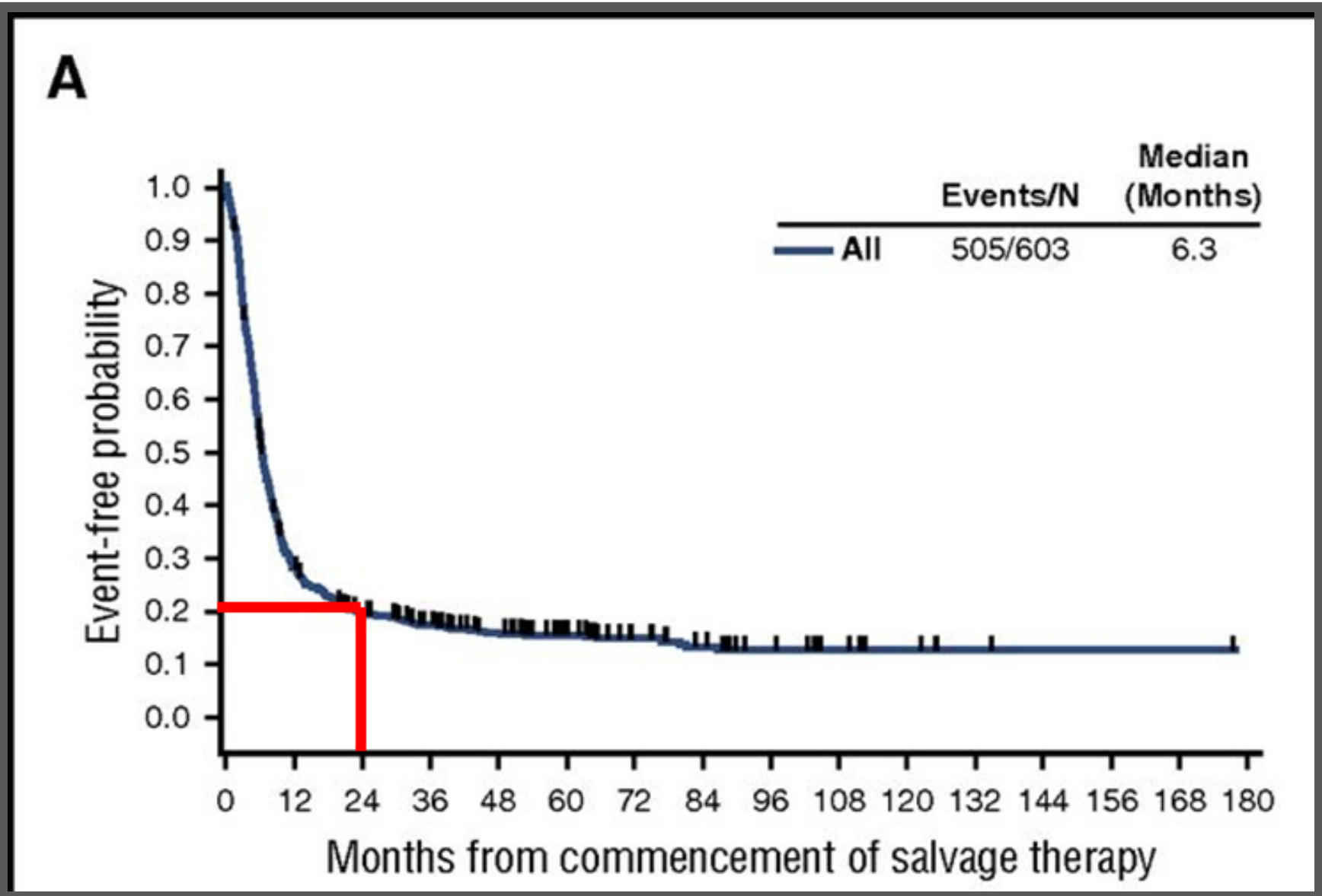
CAR – T Product	Indication
Axi-cel	R/R LBCL R/R Follicular Lymphoma (3/2021) Transformed Follicular Lymphoma R/R Primary Mediastinal B-cell Lymphoma
Tisa-cel	R/R LBCL Relapsed B-cell ALL <25 year of age Transformed low grade lymphoma except CLL
Liso-cel	R/R LBCL (2/2021) Transformed low grade NHL including FL, CLL and mariginal zone lymphoma
Brexu-cel	R/R Mantle Cell Lymphoma (up to 5 lines of therapy) Relapsed B-ALL (adult)
Ide-cel Ciltabtagene autoleucel	R/R MM (more than 4 lines of therapy) (3/2021) including a proteasome inhibitor (PI), an immunomodulatory agent (IMiD), and an anti-CD38 monoclonal antibody.

Why CAR T ?

Let us look at SCHOLAR-1:

Refractory DLBCL (including subtypes PMBCL and TFL) was defined as progressive disease (received ≥4 cycles of first-line therapy) or stable disease (received 2 cycles of later-line therapy) as best response to chemotherapy or relapse ≤12 months after ASCT.

SCHOLAR-1: Overall survival from commencement of salvage therapy



Michael Crump et al. Blood 2017;130:1800-1808

How it began

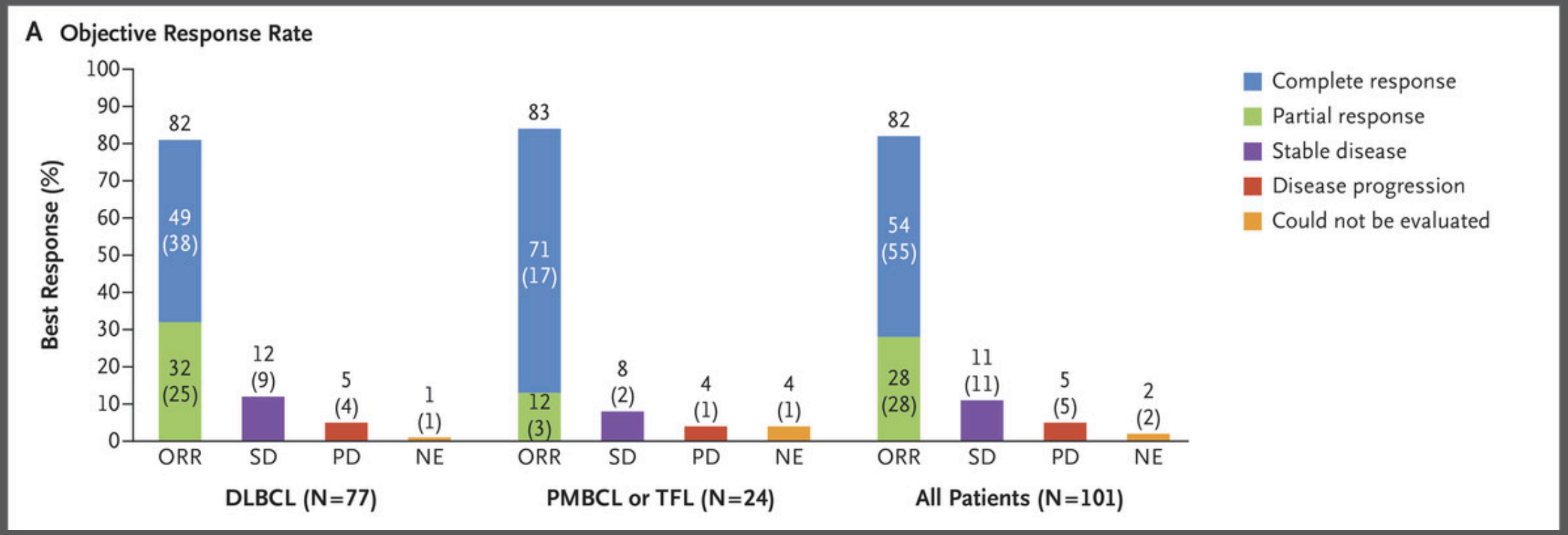
CAR-T for relapsed/refractory large B cell lymphoma

- **Refractory to second line**
- **Relapse after auto HCT**
- Historic ORR to next line of therapy 26% with CR of 7% and median OS of only 6.3 months (SCHOLAR-1 data)

Axicabtagene Ciloleucel CAR-T cell Therapy in Refractory Large B-Cell Lymphoma

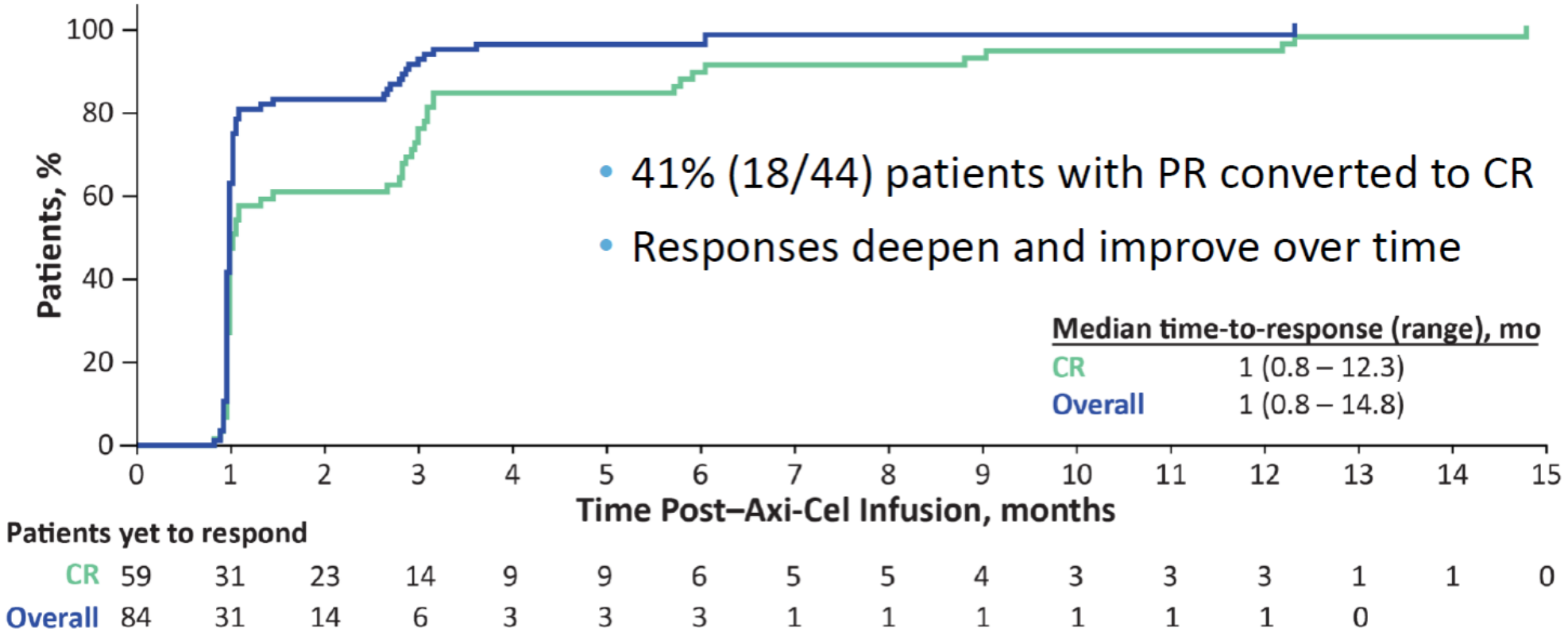
NEJM Dec 10, 2017: in second relapse or refractory to second line therapy

Objective Response Rate among the 101 Treated Patients.



Neelapu SS et al. N Engl J Med 2017;377:2531-2544.

Time to objective response and complete response



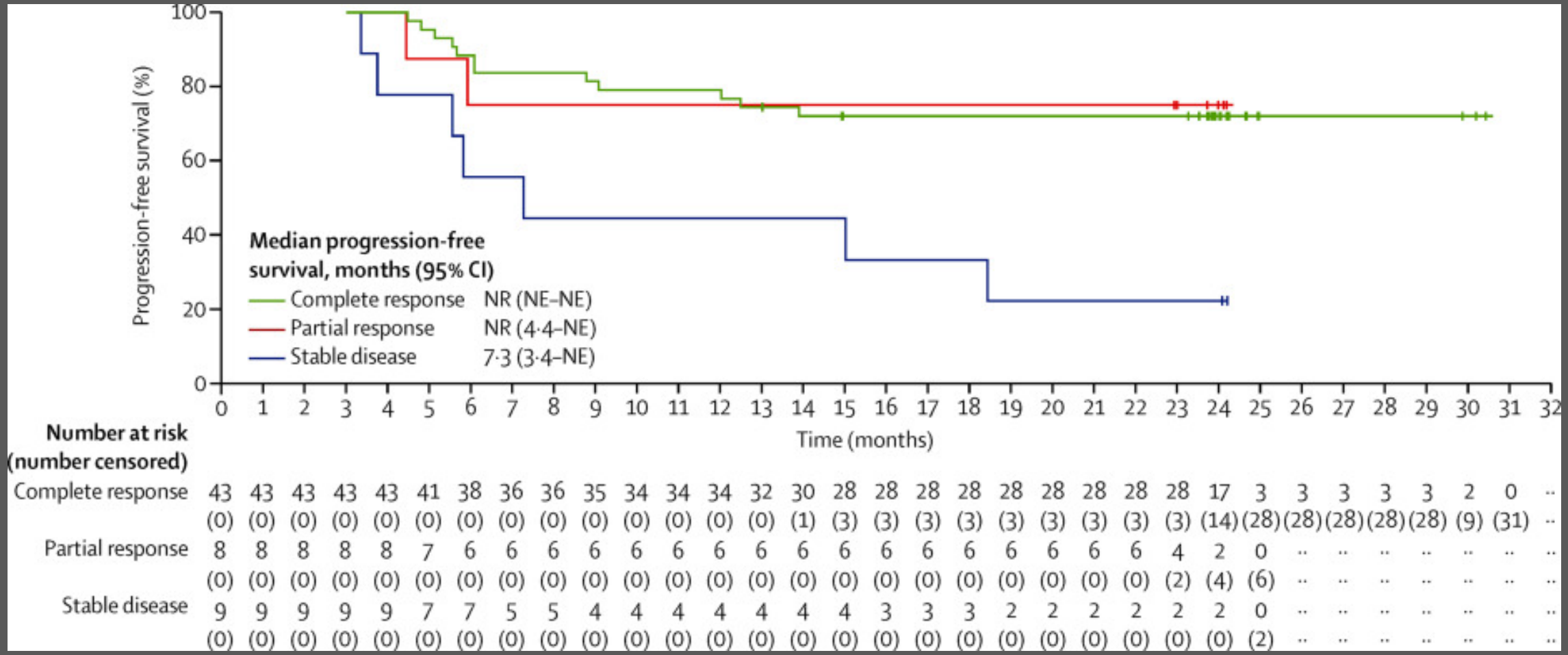
Analysis only includes those patients who achieved a response as assessed by Cheson 2007 criteria per investigator assessment.[1] First response assessment was conducted at Month 1, then every 3 months post-infusion thereafter. Time-to-response was calculated as (date of first observed response – axi-cel infusion date + 1)/(365.25/12).

1. Cheson BD, et al. *J Clin Oncol*. 2007;25:579-586.

Long-term safety and activity of axicabtagene ciloleucel in relapsed/refractory large B-cell lymphoma (ZUMA-1) in second relapse or refractory to second line therapy

Locke et al., Lancet Oncology 2019

PFS by response at month 3



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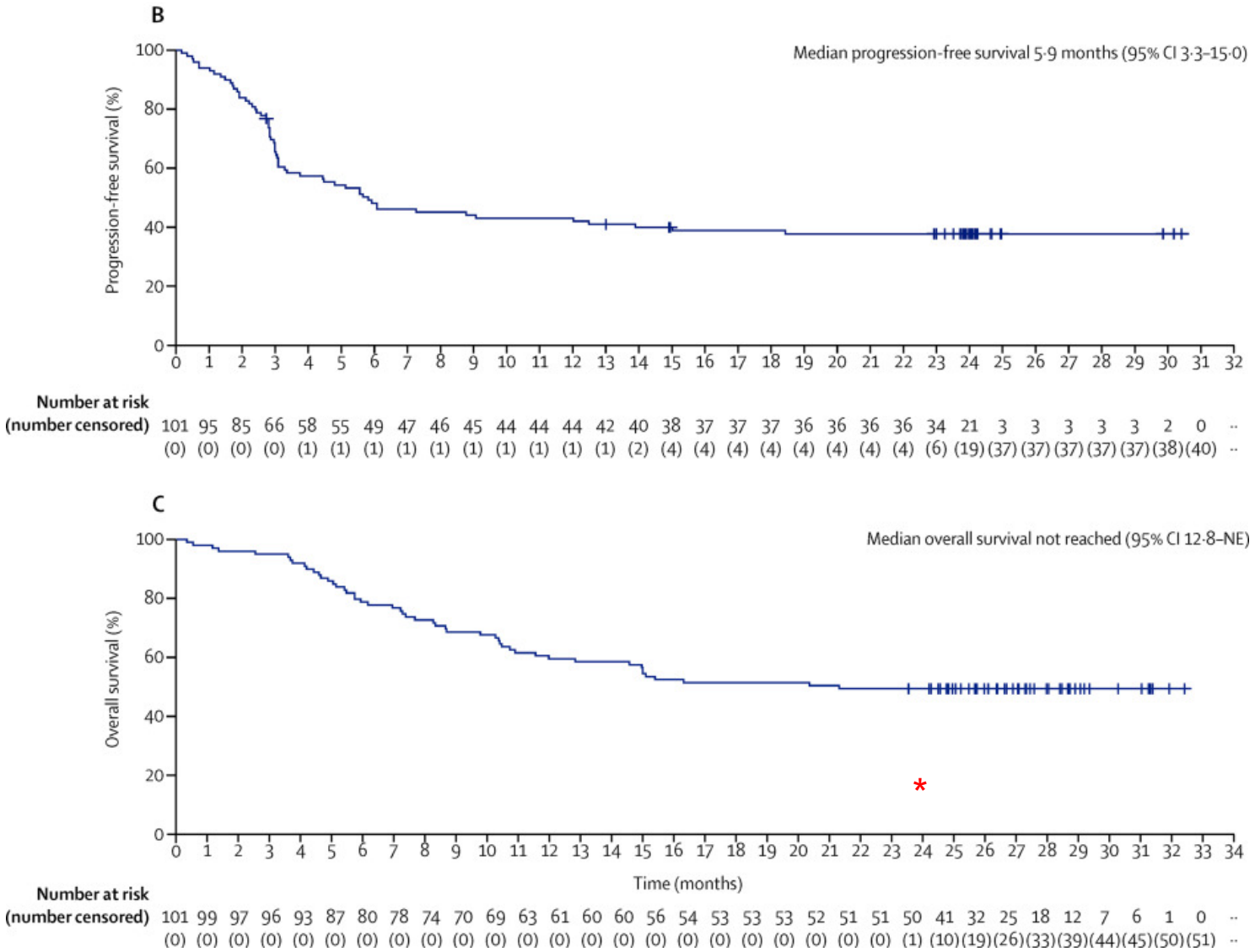
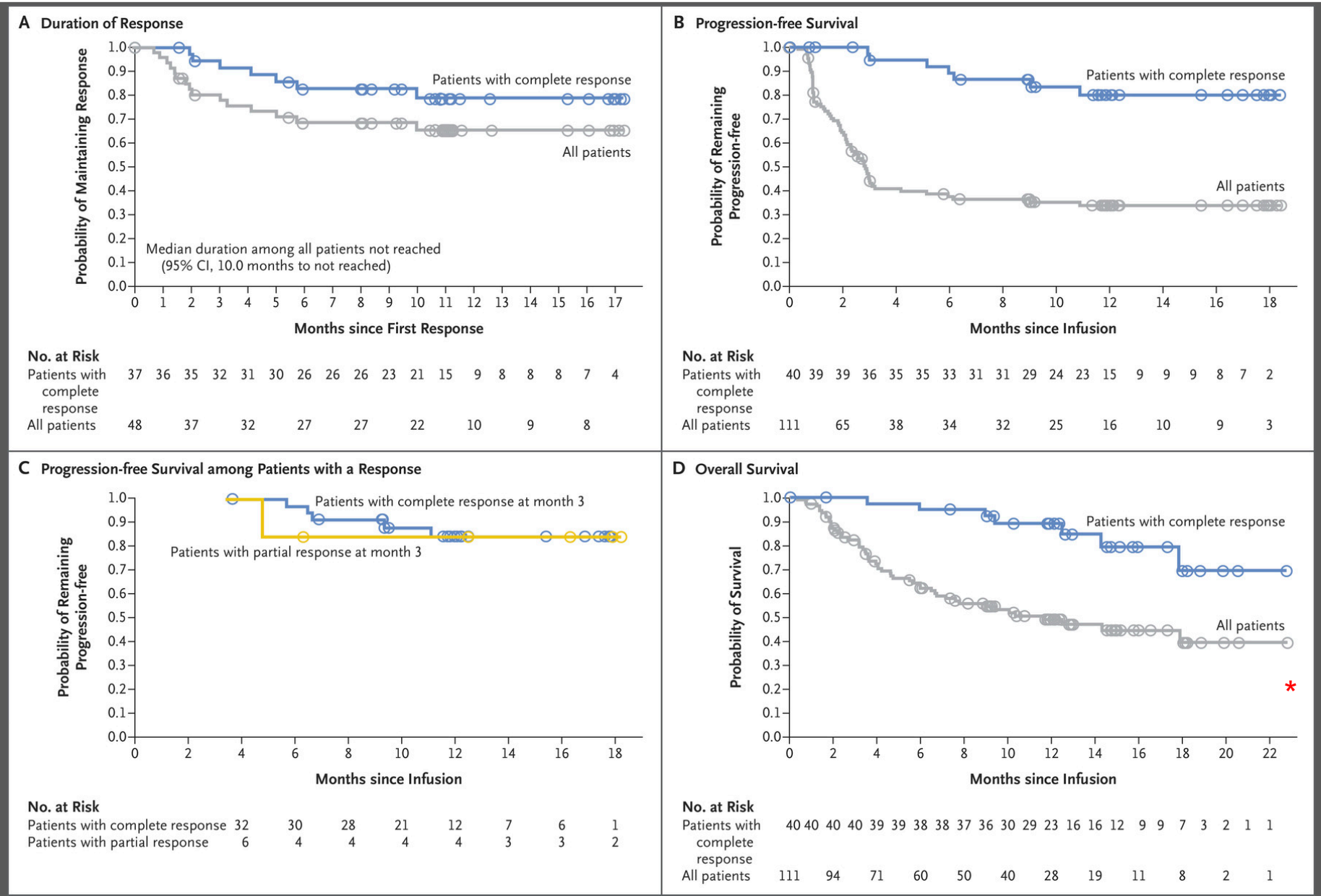


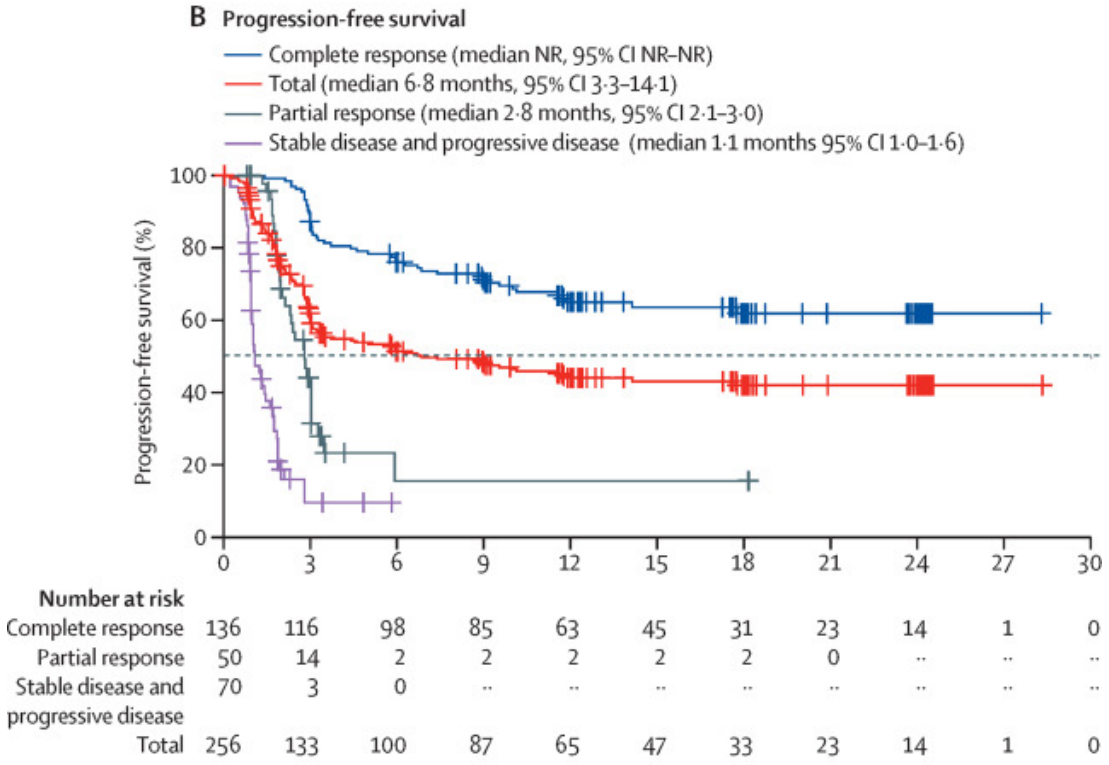
Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma (JULIET) in second relapse or refractory to second line therapy

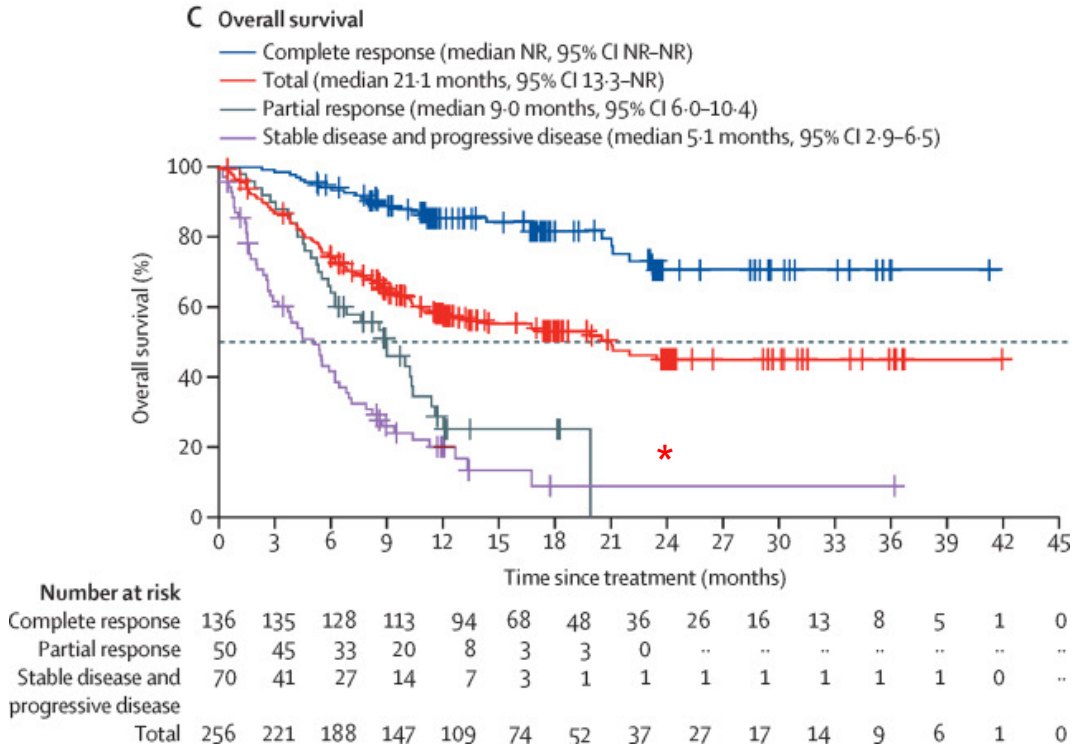
Schuster et al, NEJM 2019



Liso-cel in relapsed / refractory large B cell lymphoma: in second relapse or refractory to second line therapy

(Abramson et al., Lancet 2020)





CAR-T for relapsed/refractory large B cell lymphoma

- No obvious benefit for either one of the three commercially available CAR –T over the other for disease refractory to second line therapy or for 2nd relapse/third line treatment

Where it stands now:

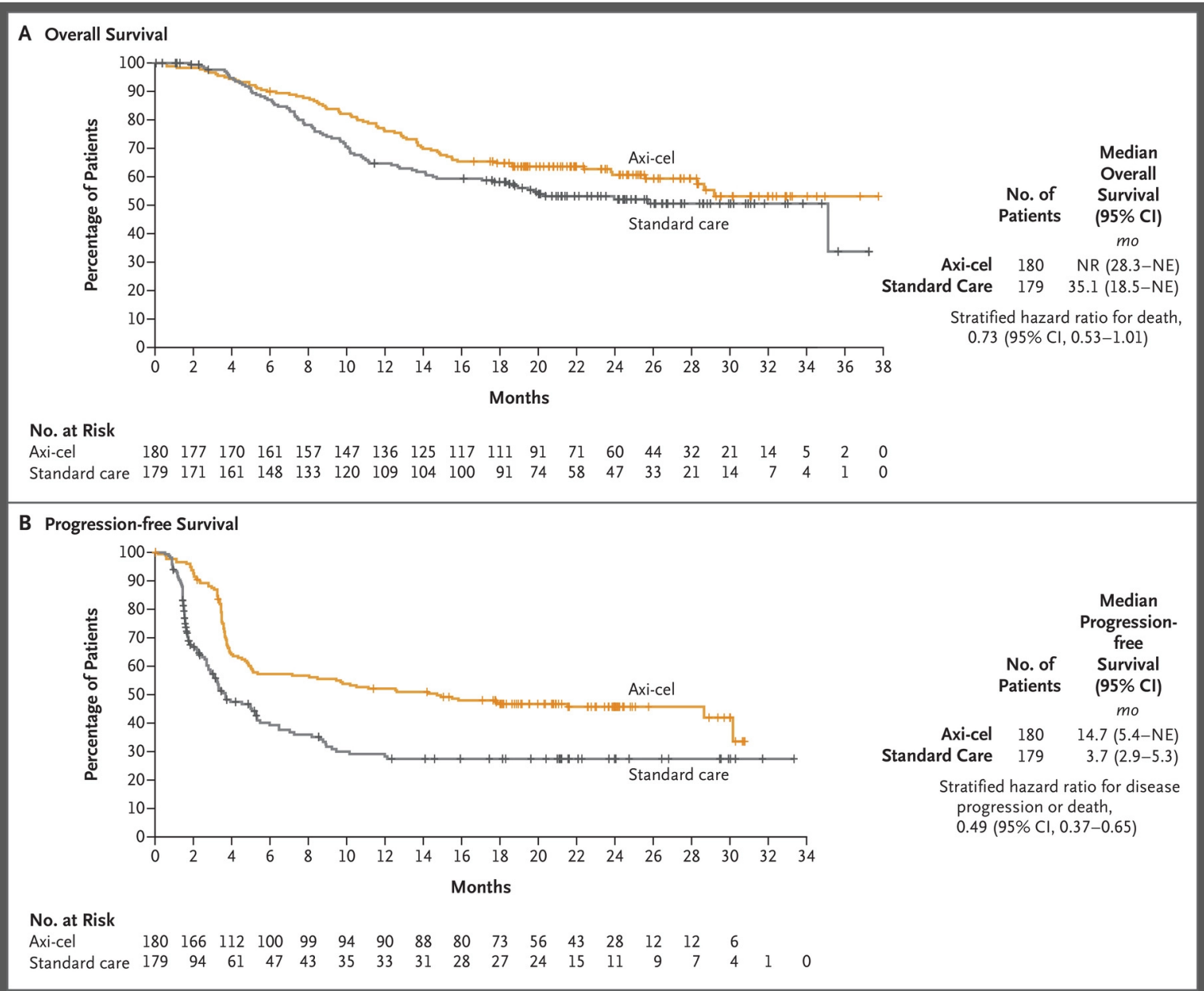
CAR-T in 2nd line for large B cell lymphoma

- **Nearly one-third** of patients relapse after achieving a complete response of diffuse large B cell lymphoma (DLBCL) using **first line** R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) and 10 percent are refractory to initial therapy.

- *Is it meaningful to bring CAR-T into earlier line of treatment, e.g., second line for relapsed/refractory disease ?*

Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma (ZUMA-7)

(Locke et al., NEJM 2022)



ASCO 2023:

47.2 months median follow up

Axi-cel: median OS not reached

SOC: median OS 31 months

Axi-cel: median PFS 14.7 months

SOC: median PFS 3.7 months

Axi-cel: 4 yr PFS 41.8%

SOC: 4 yr PFS 24.4%

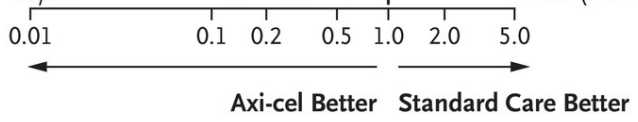
57% of SOC arm went on to receive Axi-cel

Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma (ZUMA-7)

(Locke et al., NEJM 2022)

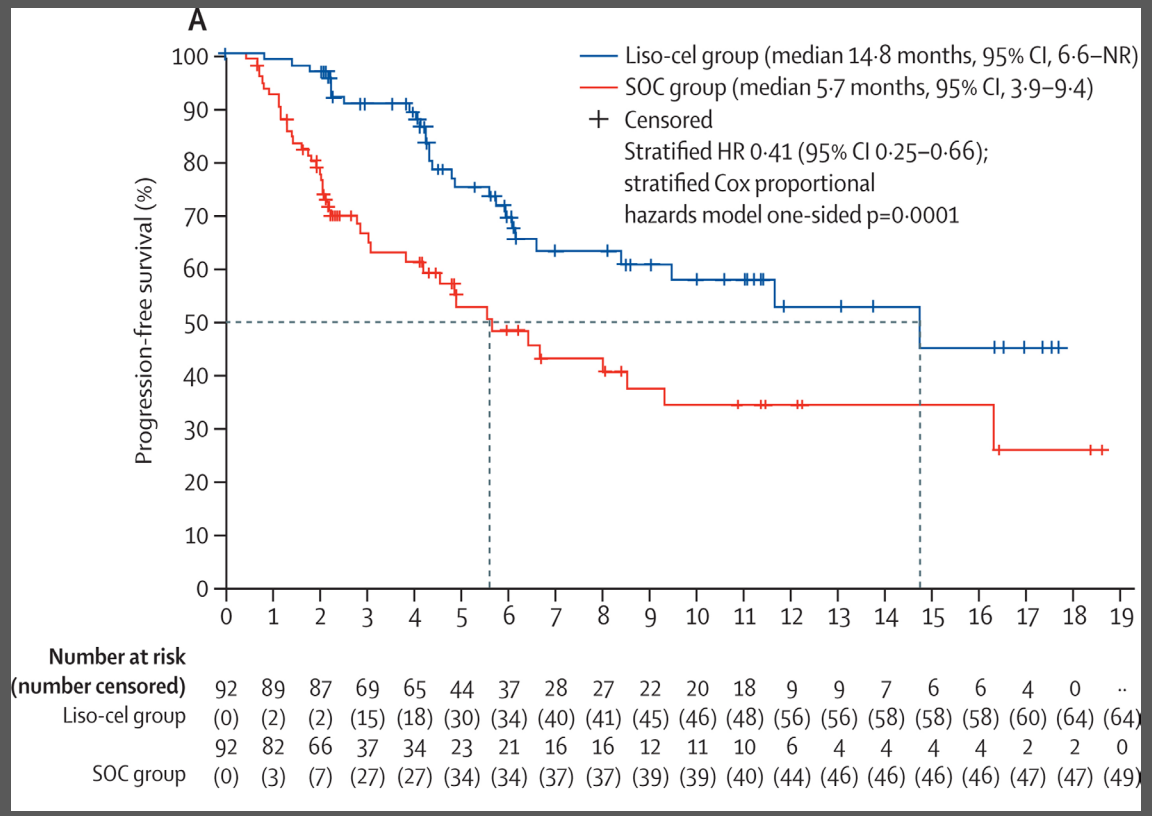
B Subgroup Analysis

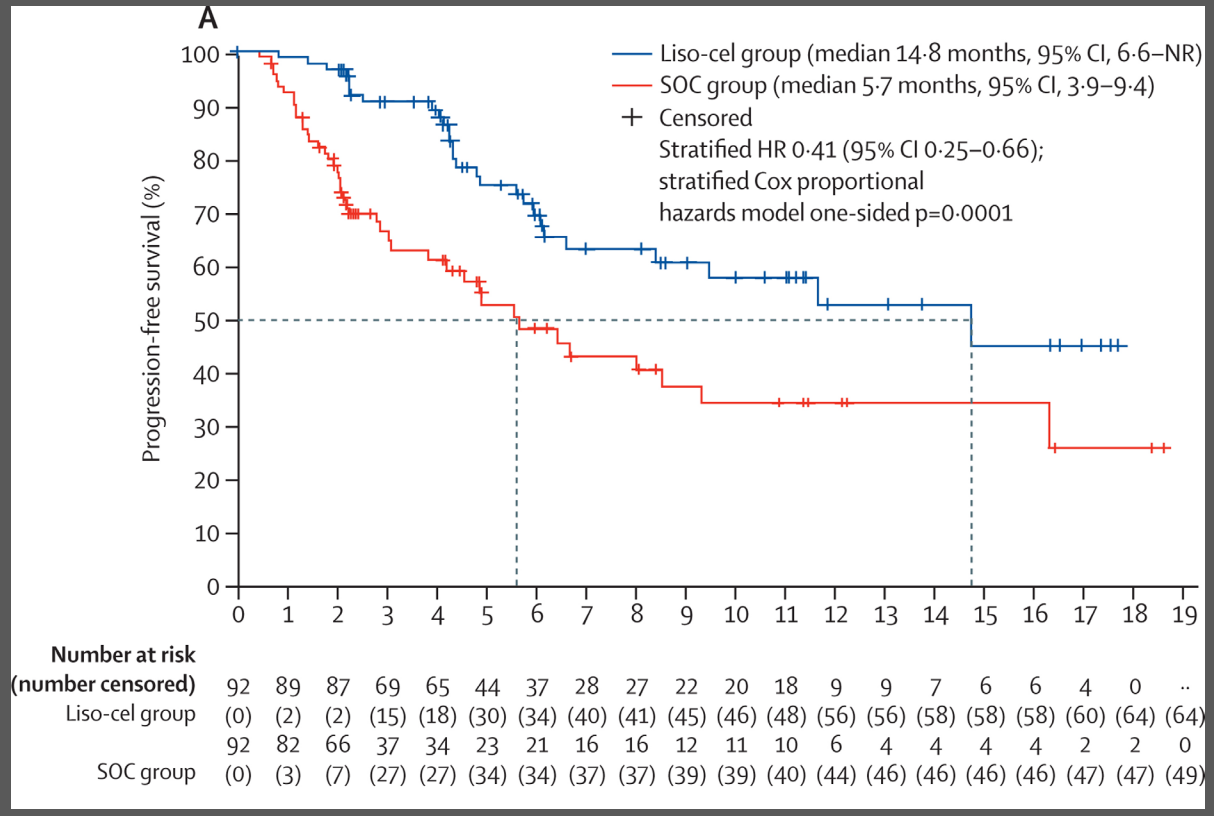
Subgroup	Axi-cel	Standard Care	Hazard Ratio for Event or Death (95% CI)
	no. of patients with event/total no.		
Overall	108/180	144/179	0.40 (0.31–0.51)
Age			
<65 yr	81/129	96/121	0.49 (0.36–0.67)
≥65 yr	27/51	48/58	0.28 (0.16–0.46)
Response to first-line therapy at randomization			
Primary refractory disease	85/133	106/131	0.43 (0.32–0.57)
Relapse ≤12 mo after initiation or completion of first-line therapy	23/47	38/48	0.34 (0.20–0.58)
Second-line age-adjusted IPI			
0 or 1	54/98	73/100	0.41 (0.28–0.58)
2 or 3	54/82	71/79	0.39 (0.27–0.56)
Prognostic marker according to central laboratory			
HGBL, double- or triple-hit	15/31	21/25	0.28 (0.14–0.59)
Double-expressor lymphoma	35/57	50/62	0.42 (0.27–0.67)
Molecular subgroup according to central laboratory			
Germinal center B-cell–like	64/109	80/99	0.41 (0.29–0.57)
Activated B-cell–like	11/16	9/9	0.18 (0.05–0.72)
Unclassified	8/17	12/14	—
Disease type according to investigator			
DLBCL, not otherwise specified	68/110	97/116	0.37 (0.27–0.52)
Large-cell transformation from follicular lymphoma	10/19	24/27	0.35 (0.16–0.77)
HGBL, including rearrangement of *MYC* with *BCL2* or *BCL6* or both	23/43	18/27	0.47 (0.24–0.90)
Disease type according to central laboratory			
DLBCL	79/126	95/120	0.44 (0.32–0.60)
HGBL, including rearrangement of *MYC* with *BCL2* or *BCL6* or both	15/31	21/26	0.28 (0.14–0.59)



Second-Line Lisocabtagene or Standard Care as Second Line in large B-Cell Lymphoma: Transform

(Kamdar M et al., The Lancet 2022)





CAR T-Cell Therapy for Large B-Cell Lymphoma — Who, When, and How?

- The ZUMA-7 trial shows that ASCT-eligible patients with relapsed or refractory large B-cell lymphoma **whose disease is controllable with glucocorticoid bridging therapy alone should be prioritized for axicabtagene ciloleucel over ASCT as second-line therapy.**

- The **TRANSFORM** trial enrolled a **broad patient population with poor prognostic features (73% had primary refractory disease and 23% had high-grade B-cell lymphoma with rearrangements of MYC and BCL2, BCL6 , or both)**, including patients with high tumor burden and rapidly progressing disease as demonstrated by the need for bridging therapy in 63% of patients, which is more representative of the real-world patient population.

- Axi-cel and liso-cel **FDA approved** as second line for large B cell lymphoma

- **Current approach: relapse within 6 months: CAR T if eligible, if beyond 6 months: second line chemotherapy and autologous HCT**

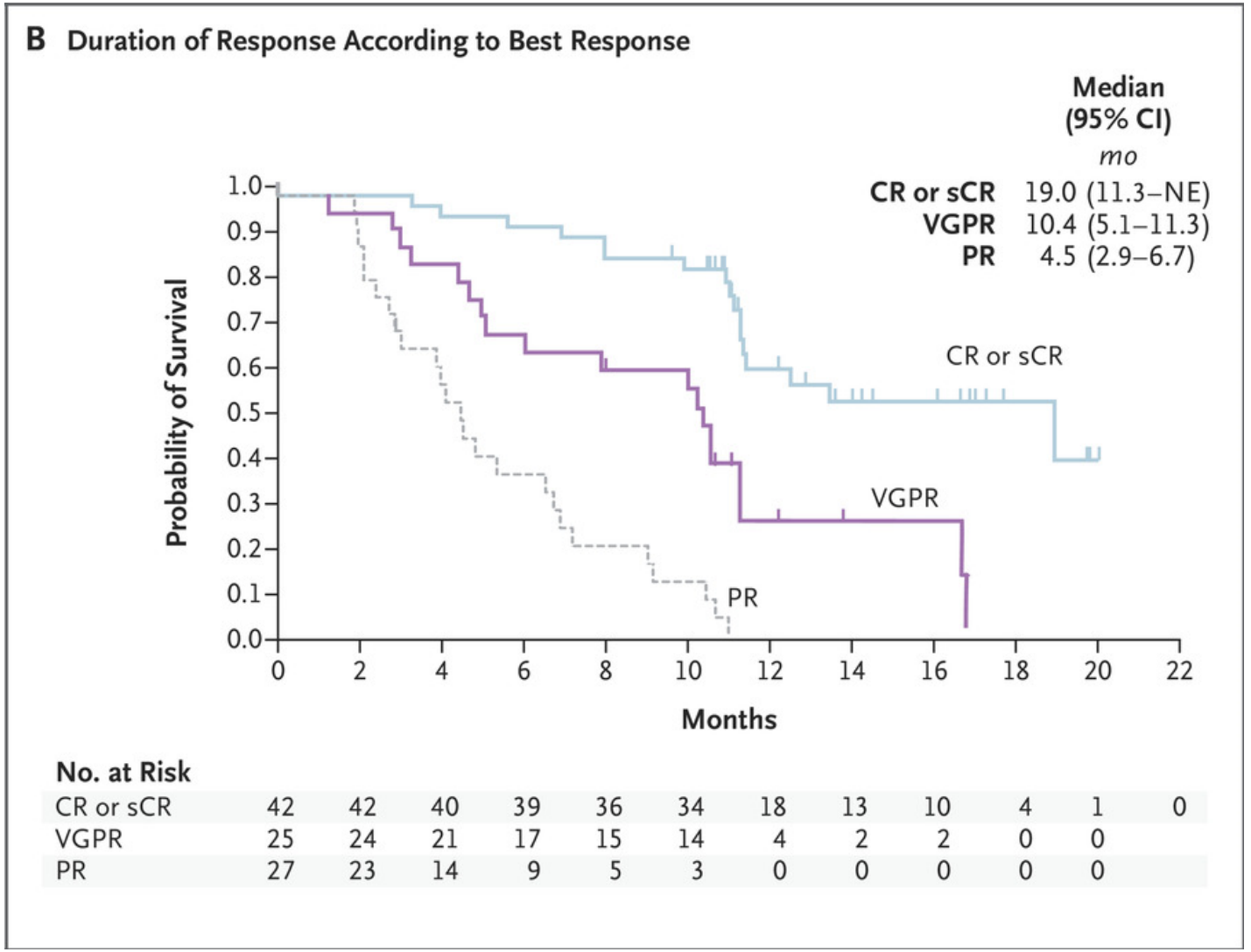
Real – world outcomes with novel therapies in R/R DLBCL

Outcome (95% CI)	Treatment in the 2L+ setting			Treatment in the 3L+ setting		
	CAR T (n=73)	**Pola-BR (n=69)**	**Tafa-len (n=27)**	**CAR T (n=55)**	**Pola-BR (n=37)**	**Tafa-len (n=20)**
ORR (%)	76.7 (65.4, 85.8)	59.4 (46.9, 71.1)	40.7 (22.4, 61.2)	74.6 (61.0, 85.3)	62.2 (44.8, 77.5)	35.0 (15.4, 59.2)
CR (%)	52.1 (40.0, 63.9)	18.8 (10.4, 30.1)	11.1 (2.4, 29.2)	41.8 (28.7, 55.9)	13.5 (4.5, 28.8)	10.0 (1.2, 31.7)
mPFS (mo)	6.7 (4.0, 10.0)	3.1 (1.9, 3.8)	1.9 (0.8, 3.5)	5.6 (2.9, 7.4)	3.4 (2.1, 4.4)	1.7 (0.7, 4.4)
mOS (mo)	26.5 (13.6, NE)	7.8 (5.6, 11.4)	6.3 (1.6, 16.2)	17.8 (9.6, NE)	7.4 (4.3, 10.9)	6.3 (1.6, 16.2)

Leigh Crombie J, et al., ASCO 2023 7552

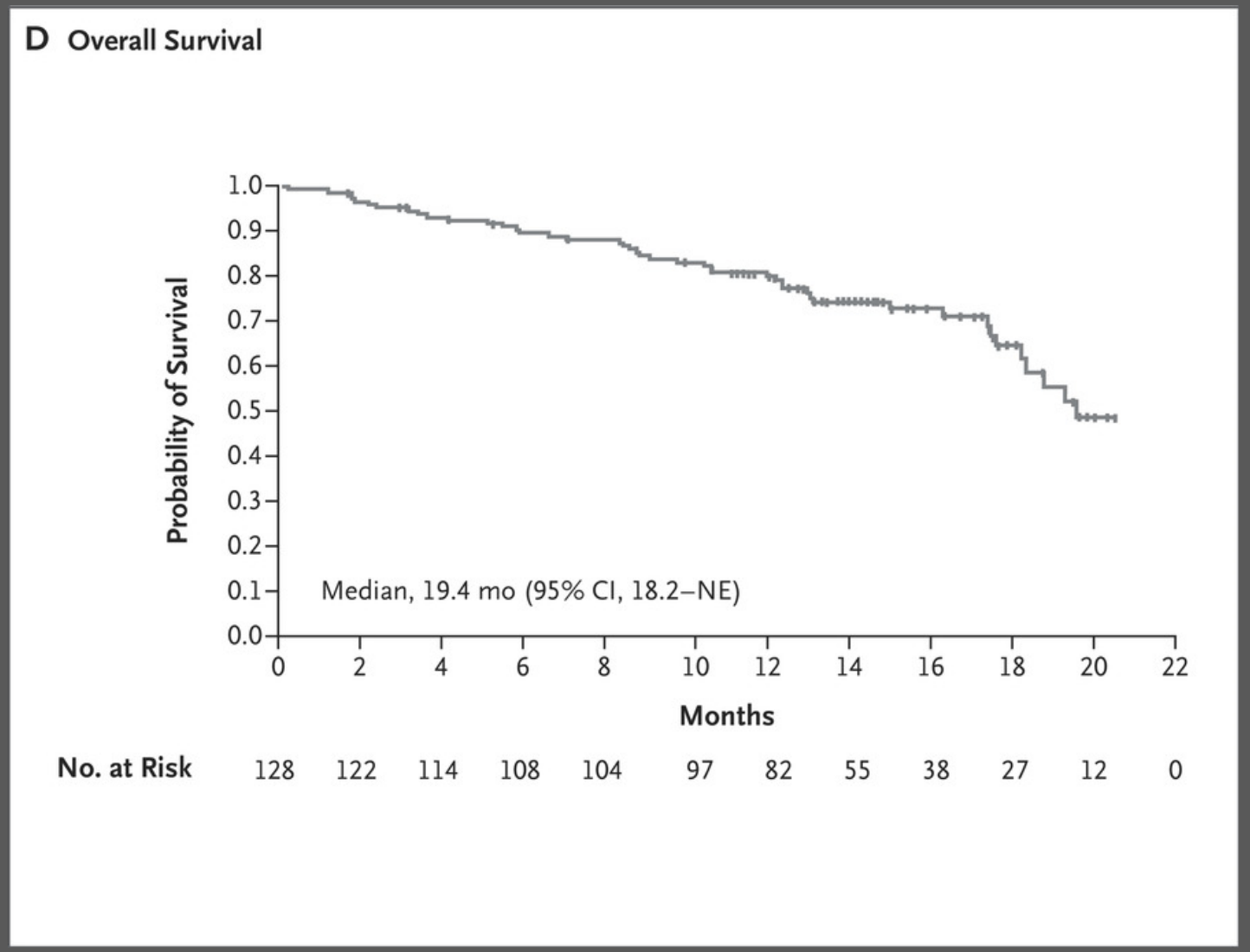
CAR T for multiple myeloma

Munshi et al. NEJM Feb 2021



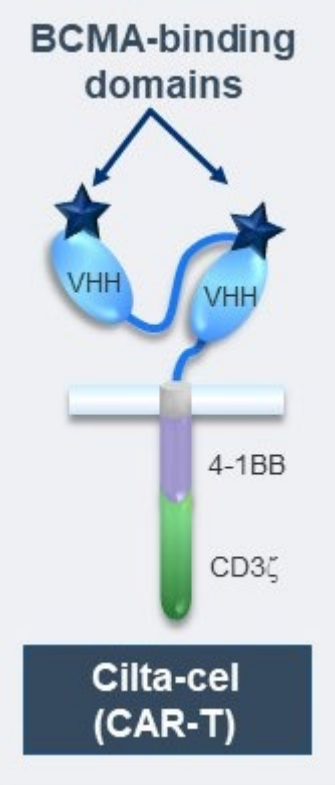
CAR T for multiple myeloma

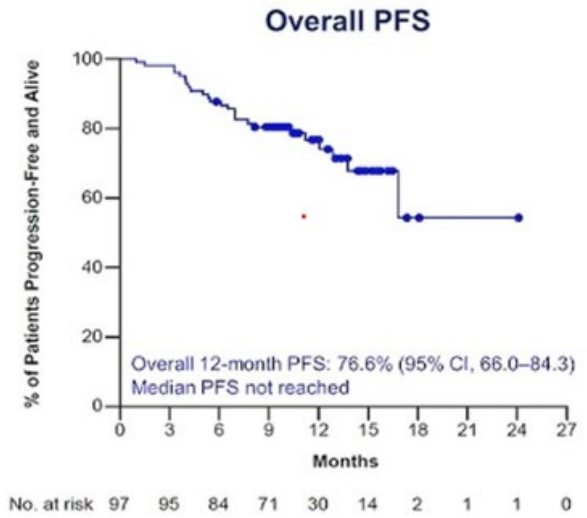
Munshi et al. NEJM Feb 2021: Ide-cel

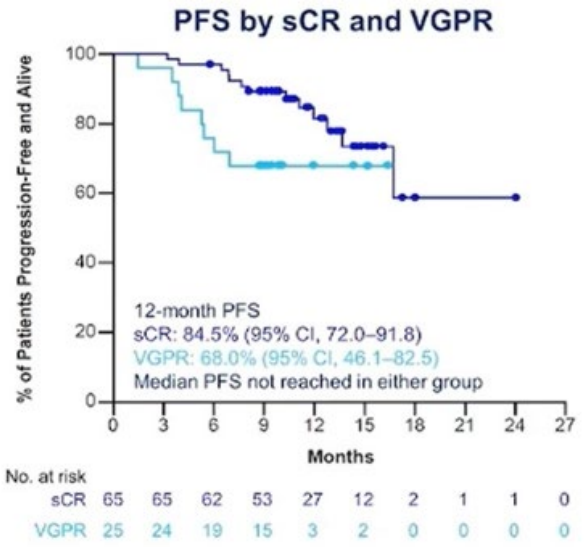


CAR T for multiple myeloma

- Berdeja et al (2021) *The Lancet: Ciltacel*







ASH 2021 update:

- Responses deepened over time from 1 year follow up
- sCR at 1 year was 67% and at 2 years is 82.5%
- Median time to best response was 2.6 months
- Median time to CR or better was 2.9 months
- Median duration of response was not estimable
- OS 74%
- 60.5% of patient are still PF at 2 years
  - sCR PFS of 71%

CAR T for multiple myeloma

CARTITUDE-4 update ASCO 2023:

- cilta-cel versus SOC (PVd, DPd) in lenalidomide refractory patients

Cilta-cel vs SOC outcomes (ITT).

	Cilta-cel (n=208)	SOC (n=211)	HR[a]	Odds ratio
Median PFS, mo (95% CI)	NE (23–NE)	12 (10–14)	0.26 (0.18–0.38) (*P*<0.0001)	
12-mo PFS, % (95% CI)	76 (69–81)	49 (42–55)		
ORR, n (%)[b]	176 (85)	142 (67)		3 (*P*<0.0001)
≥CR[b]	152 (73)	46 (22)		10 (*P*<0.0001)
10^{-5} MRD negative,[c] n (%)	126 (61)	33 (16)		9 (*P*<0.0001)

[a]Per computerized algorithm by constant piecewise weighted log-rank test. [b]In 176 pts who received cilta-cel as study tx: ORR, 175 (99%); ≥CR, 152 (86%). [c]For MRD-evaluable pts: cilta-cel, 88% (126/144); SOC, 33% (33/101).

ASCO 2023

Major complications of CAR-T cell therapy:

- **Cytokine release syndrome (CRS)**
  - Incidence and severity dependent on different factors
    - Costimulatory domain
    - Number of cells infused
    - Ratio of CD4/CD8
    - Disease burden
  - Fever, chills, shortness of breath (“looks like bacteremia / sepsis or ARDS”)
  - **Onset: early day 2-7, after that much more rare**
  - **Treatment: tocilizumab plus/minus steroids**

- **Immune effector cell-associated neurotoxicity syndrome (ICANS)**
  - Similar risk factors as CRS
  - Symptoms can vary substantially: speech problems, confusion, drowsiness, stupor, coma, vision problems, memory difficulties
  - Slightly later onset, up to day 60
  - **Treatment: dexamethasone (plus tocilizumab if CRS present)**

Major complications of CAR-T cell therapy:

- Prolonged cytopenia: usually resolves within a month, but can persist, evaluate for clonal hematopoiesis, MDS, consider growth factor support

- Hypogammaglobulinemia / B cell aplasia (with B-/Plasma cell directed CAR-T): check IgG level every 4 weeks, substitute if IgG <400mg/dl

- PCP prophylaxis and Herpes viridae prophylaxis

- CAR-T cell derived T cell lymphoma

CAR T overview

Product	Axicabtagene Ciloleucel	Brexucabtagene autoleucel	Tisagenlecleucel	Lisocabtagene maraleucel	Idecabtagene vicleucel
Commercial Name	(Yescarta®)	(Tecartus®)	(Kymriah®)	(Breyanzi®)	(Abecma™)
Synonyms	KTE-C19	KTE-X19	JCAR017	JCAR017	Bb2121
Pharmaceutical Company	Kite	Kite	Novartis	Bristol-Myers Squibb	Bristol-Myers Squibb
Indication(s)	See full prescribing information	See full prescribing information	See full prescribing information	See full prescribing information	See full prescribing information
Target	CD19	CD19	CD19	CD19	BCMA
Costimulatory Domain	CD28	CD28, CD3-zeta	4-IBB	4-1BB, CD3-zeta	4-1BB
Viral Vector	Retroviral	Retroviral	Lentiviral	Lentiviral	Lentiviral
Lymphodepletion	Cyclophosphamide 500mg/m^2 Fludarabine 30mg/m^2 Day -5, -4, -3	Cyclophosphamide 500mg/m^2 Fludarabine 30mg/m^2 Day -5, -4, -3	Cyclophosphamide 250mg/m^2 Fludarabine 25mg/m^2 x 3 days	Cyclophosphamide 300mg/m^2 Fludarabine 30mg/m^2 X 3 days	Cyclophosphamide 300mg/m^2 Fludarabine 30mg/m^2 x 3 days
Cell Infusion Timing	3 days after completion of lymphodepletion chemotherapy	3 days after completion of lymphodepletion chemotherapy	2-11 days after completion of lymphodepletion chemotherapy	2 days after completion of lymphodepletion chemotherapy	2 days after completion of lymphodepletion chemotherapy
Target Dose (T cells/kg)	2 x 10^6	2 x 10^6 – 2 x 10^8	0.6-6 x 10^8	300-460 x 10^6 (Total T cells; not per kg)	300-460 x 10^6 (Total T cells; not per kg)
Toxicity Onset (median, range)	CRS (2 days, 1-12) Neurotoxicity (4 days, 1-43)	CRS (3 days, 1-13) Neurotoxicity (6 days, 1-32)	CRS (3 days, 1-51) Neurotoxicity (6 days, 1-359)	CRS (5 days, 1-15) Neurotoxicity (8 days, 1-46)	CRS (1 day, 1-12) Neurotoxicity (2 days, 1-10)
Toxicity Duration (median, range)	CRS (7 days, 2-58) Neurotoxicity (17 days)	CRS (10 days, 1-50) Neurotoxicity (21 days, 2-454)	CRS (8 days, 1-36) Neurotoxicity (ALL: 6 days; DLBCL: 14 days)	CRS (5 days, 1-30) Neurotoxicity (15 days, 1-785)	CRS (5 days, 1-63) Neurotoxicity (3 days, 1-26)

CRS management

CRS Grade	Management with Tocilizumab	Management with Corticosteroids
Grade 1 **Symptoms are not life threatening and require symptomatic treatment only (ie. Fever, nausea, fatigue, headache, malaise)**	Supportive care and rule out infection Axicabtagene ciloluecel or brexucabtagene autoleucel: if symptoms (e.g., fever) not improving after 24 hours, consider tocilzumab IV once. Idecabtagene vicleucel or lisocabtagene maraleucel: if symptoms occur < 72 hours after infusion, consider tocilizumab IV once, If > 72 hours after infusion, treat symptomatically. Tisagenlecleucel: If persistent (>3 days) or refractory fever, consider tocilizumab IV once.	Axicabtagene ciloluecel: if symptoms not improving after 3 days, administer dexamethasone 10 mg IV once. Idecabtagene vicleucel or lisocabtagene maraleucel: if symptoms occur <72 hours after infusion, consider dexamethasone 10 mg IV every 24 hours. If > 72 hours after infusion, treat symptomatically. Brexucabtagene autoleucel or tisagenlecleusel: not indicated
Grade 2* **Symptoms require and respond to moderate intervention** • **Oxygen requirement ≤6L/min nasal cannula or blow-by** • **Hypotension not requiring vasopressors**	Administer tocilizumab IV once (preferred) or siltuximab IV once (if tocilizumab unavailable): may repeat dose every 8 hours. Limit anti -IL-6 therapy to a maximum of 3 doses in 24 hours and up to 4 total doses of anti -IL-6 therapy	If no improvement within 24 hours after stating anti-IL-6 therapy: administer dexamethasone 10 mg IV every 6 hours. Continue until Grade 1 or less, taper over 3 days
Grade 3* **Symptoms require and respond to aggressive intervention** • **Oxygen requirement >6L/min nasal cannula, facemask, nonrebreather mask, or Venturi mask** • **Hypotension requiring vasopressor with or without vasopressin**	Administer tocilizumab IV once (preferred) or siltuximab IV once (if tocilizumab unavailable): may repeat dose every 8 hours. Limit anti -IL-6 therapy to a maximum of 3 doses in 24 hours and up to 4 total doses of anti -IL-6 therapy	Administer dexamethasone 10 mg IV every 6 hours. Continue until Grade 1 or less, taper over 3 days
Grade 4* **Life threatening symptoms:** • **Requiring positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)** • **Requiring multiple vasopressors (excluding vasopressin)**	Administer tocilizumab IV once (preferred) or siltuximab IV once (if tocilizumab unavailable): may repeat dose every 8 hours. Limit anti -IL-6 therapy to a maximum of 3 doses in 24 hours and up to 4 total doses of anti -IL-6 therapy	Administer high-dose methylprednisolone 1000 mg IV every 24 hours for 3 days. If improves, manage as above. Continue until Grade 1 or less, taper as appropriate

[1] See Table 2 for symptom definitions and supportive care interventions
[2] Siltuximab 11 mg/kg. Patient consent must be documented prior to siltuximab administration.

Supportive measures

Sign/Symptom	Supportive Care Management
Fever **Single oral temperature ≥38.3°C (101.0°F)** **OR** **Temperature of ≥38.0°C (100.4°F) for ≥1 hour or twice in 24 hours**	• Reference Physician Orders - Ped HEM ONC Fever Neutropenia Admit - Downtime Orders • Obtain blood cultures x 2 sets(peripheral and from each lumen of central venous catheter • CBC with diff, CMP, chest x-ray • Site-specific cultures (as symptoms dictate) • Stool: C.difficile PCR if diarrhea present (Only consider GI panel if admission <72 hours and suspect enteric pathogen) • CSF: suspected meningitis • Respiratory: sputum sample for routine culture if productive cough • Skin: aspiration or biopsy of suspected skin lesions • Urine: urinalysis and urine culture if signs or symptoms of a urinary tract infection (UTI) • Activate conditional anti-pseudomonal antibiotic order STAT • Piperacillin-tazobactam preferred for CAR-T-cell patients • Aztreonam + vancomycin for serious beta-lactam allergies (e.g., hives, anaphylaxis) • Consider addition of additional antibiotic coverage based on history, infectious work-up, and clinical manifestations • Acetaminophen may be given as a single dose on an as-needed basis. For repeat dosing, contact provider
Neutropenia **Absolute neutrophil count <0.5x 10^9/L (or 500 cells/µL**	May consider growth factor support with filgrastim for patients with neutropenia lasting ≥ 7 days
Nausea and/or vomiting	Prochlorperazine 10mg PO/IV every 6 hours as needed
Hypoxia **Requiring supplemental oxygen to maintain oxygen saturation greater than 90%**	• Use supplemental oxygen as needed (nasal cannula preferred first line) • Consider high-flow oxygen delivery or non-invasive positive pressure ventilation if needed • For hypoxia unresponsive to interventions: Contact ICU for consideration of mechanical ventilation
Hypotension **Systolic blood pressure (SBP) less than 90mmHg**	IV fluid bolus of 1000ml normal saline STAT; repeat as needed to maintain SBP greater than 90mmHg For fluid-refractory hypotension: Contact ICU for consideration of vasopressor therapy. Obtain ECHO.

ICANS management

[1] See Appendix A for ICE 10-Point Neurological Assessment and Grading
[2] Siltuximab 11 mg/kg. Patient consent must be documented prior to siltuximab administration.

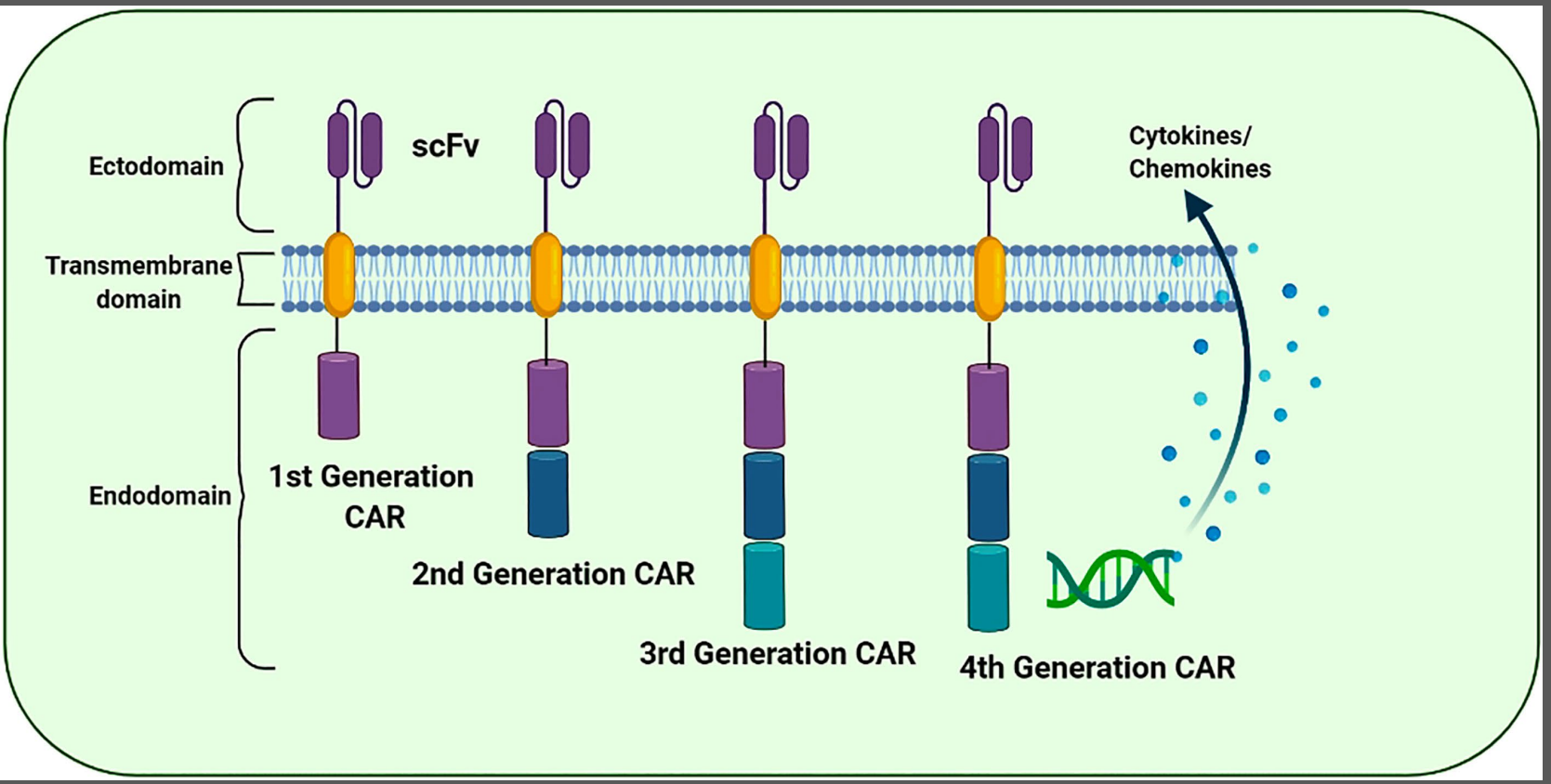
ICANS grade	Management (Concurrent CRS)	Management (No Concurrent CRS)
Grade 1 **ICE Score= 7-9**	Supportive care and rule out infection	
	Axicabtagene ciloluecel: CRS treatment per Table 1. Consider dexamethasone 10mg IV once. May repeat one dose of dexamethasone if not improving after 2 days. brexucabtagene autoleucel: CRS treatment per Table 1. Idecabtagene vicleucel or lisocabtagene maraleucel: CRS treatment per Table 1. If symptoms occur< 72 hours after infusion, consider dexamethasone 10mg IV every 12-24 hours for 2-3 days. If >72 hours after infusion, treat symptomatically Tisagenlecleucel: CRS treatment per Table 1. Consider addition of dexamethasone if additional tocilizumab doses required	Axicabtagene ciloluecel: Consider dexamethasone 10 mg IV once. May repeat one dose of dexamethasone if not improving after 2 days Idecabtagene vicleucel or lisocabtagene maraleucel: if symptoms occur <72 hours after infusion, consider dexamethasone 10 mg IV every 12-24 hours for 2-3 days. If > 72 hours after infusion, treat symptomatically. Brexucabtagene autoleucel or tisagenlecleusel: supportive care only
Grade 2 **ICE Score=3-6** **Symptoms include:** • **Somnolence – moderate, limiting instrumental DLS** • **Confusion – moderate disorientation** • **Encephalopathy – limiting instrumental ADLs** • **Dysphasia – moderate impairing ability to communicate spontaneously** • **Seizure(s)**	Administer tocilizumab or siltuximab as per Table 1 (Management of CRS) If no improvement within 24 hours after starting anti -IL-6 therapy: administer dexamethasone 10mg IV every 6 hours. Continue until Grade 1 or less, taper over 3 days.	Administer dexamethasone 10 mg IV every 6 hours. Continue until Grade 1 or less, taper over 3 days
Grade 3 **ICE score=0-2** **Symptoms include** • **Somnolence – obtundation or stupor** • **Confusion – severe disorientation** • **Encephalopathy – limiting self-care ADLS** • **Dysphasia – severe receptive or expressive characteristics, impairing ability to read, write or communicate intelligibly**	Administer tocilizumab or siltuximab as per Table 1 (Management of CRS) Administer dexamethasone 10mg IV every 6 hours beginning with the first dose of anti-IL-6 therapy. Continue until Grade 1 or less, taper over 3 days.	Administer dexamethasone 10 mg IV every 6 hours. Continue until Grade 1 or less, taper over 3 days
Grade 4 **ICE score= 0** **Patient critical or obtunded on exam** **Life threatening consequences** • **Urgent intervention indicated** • **Requirement for mechanical intervention** • **Consider cerebral edema**	Administer tocilizumab or siltuximab as per Table 1 (Management of CRS) Administer methylprednisolone 1000mg IV every 24 hours beginning with the first dose of anti-IL-6 therapy. If improving, manage as above. Continue until Grade 1 or less, taper as appropriate	Administer methylprednisolone 1000 mg IV every 24 hours for 3 days. If improves, manage as above. Continue until Grade 1 or less, taper as appropriate

ICANS grading

Neurotoxicity Domain ‡	Grade 1	Grade 2	Grade 3	Grade 4
ICE score^	7-9	3-6	0-2	0 (patient is unrousable and unable to perform ICE)
Depressed level of Consciousness˚	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimulus 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 without 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	M/A	Deep focal motor weakness such as hemiparesis or paraparesis
Raised intracranial pressure/ 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 cushings' triad

‡ ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause. For example, a patient with an ICE score of 3 who has a generalized seizure is classified as caving Grade 3 ICANS.
^ A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia. But a patient with an ICE score of 0 may be classified as having Grade 4 ICANS if the patient is unarousable.
˚Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication)
§Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0 but they do not influence ICANS grading
 # Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAEv5.0

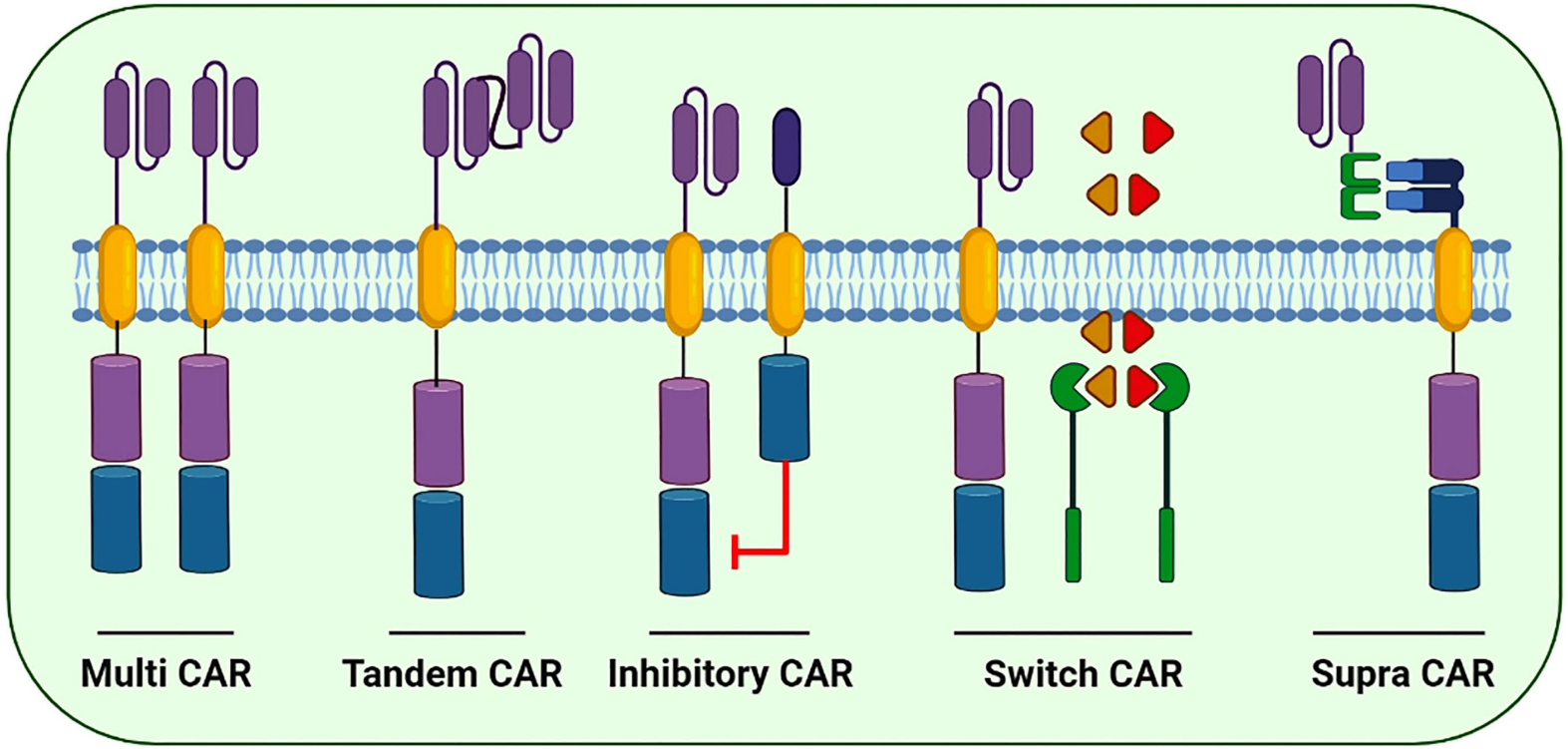
Where will the future take us



- The 1st generation of CARs failed to deliver cell proliferation signals for the retention of anti-cancer potential.
- 2nd and 3rd generation CARs have CD28, CD134 (OX40), and CD137 (4-1BB) to promote the anti-tumor potential.
- 4th CAR generation is designed to secrete cytokines to further improvise the therapeutic activity of the CAR-based immunotherapies.

Khawar et al. Frontiers in Immunology 2021

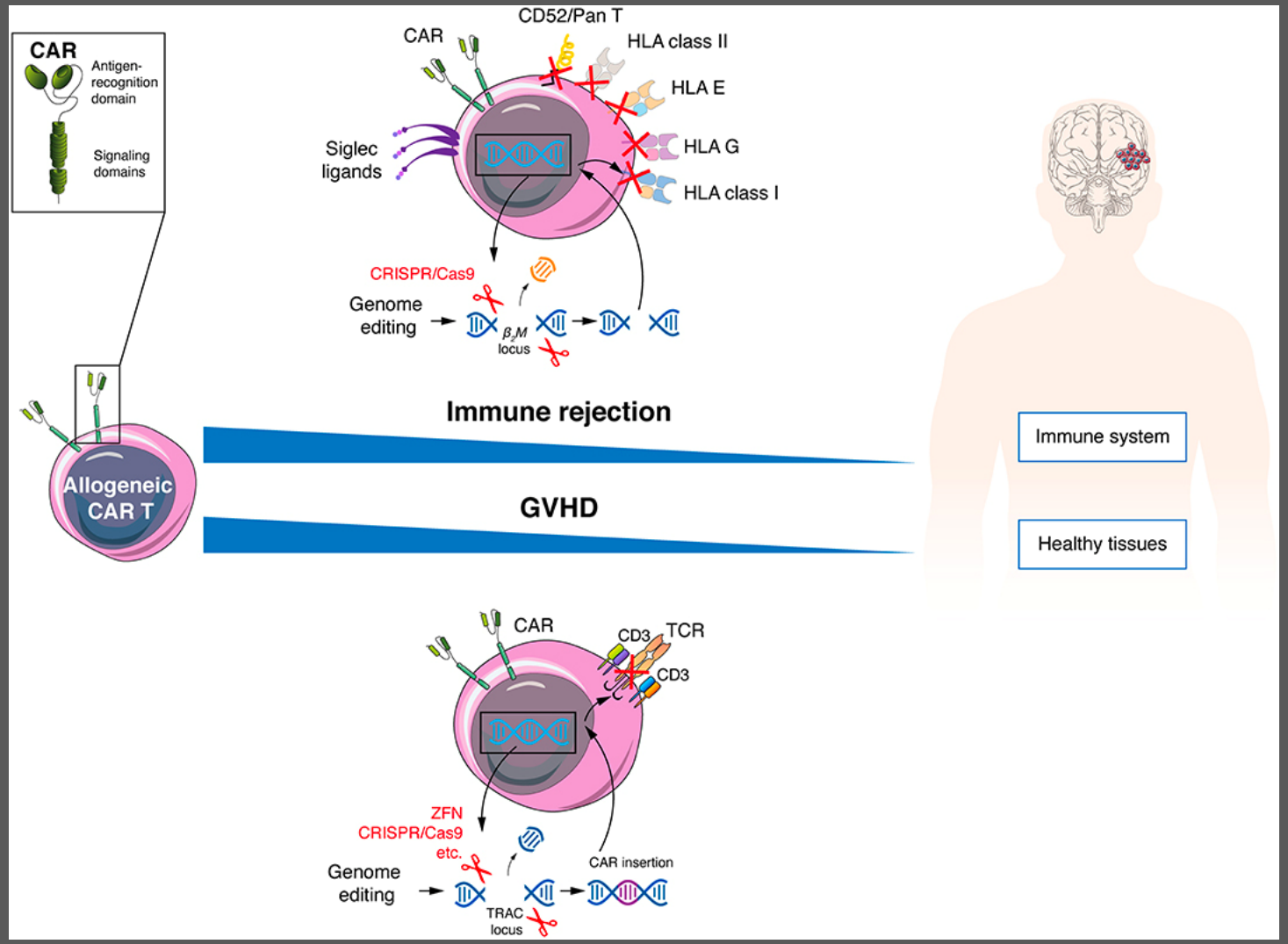
Where will the future take us



Few of the next generation CARs to better cope with the immune escape and improve the cytotoxic potential of CAR-based immunotherapies:
Multi CARs: two or more separate CARs expressing various ScFvs to target the cancer cells. Tandem CARs: two different scFvs in a single CAR molecule.
Inhibitory CARs: Upon antigen recognition in healthy cells, tend to inhibit immune cell activation.
Switch CARs: certain chemicals capable of dimerization with the iCasp9 are conditionally administered to activate the downstream caspase molecules leading to the apoptosis of CAR-expressing cells.
Supra CARs: two split structures; the antigen-binding domain (zipFV) and function domain (zipCAR) that upon binding activates the CAR-expressing cells.

Khawar et al. Frontiers in Immunology 2021

Off the shelf allo CAR T cells



Allogeneic CAR T cells
- must avoid host immune rejection and GVHD

- can evade the patient immune response by genetic disruption of HLA class I and II molecules

- resist lymphodepleting regimens using anti-CD52 antibodies by elimination of the CD52 molecule

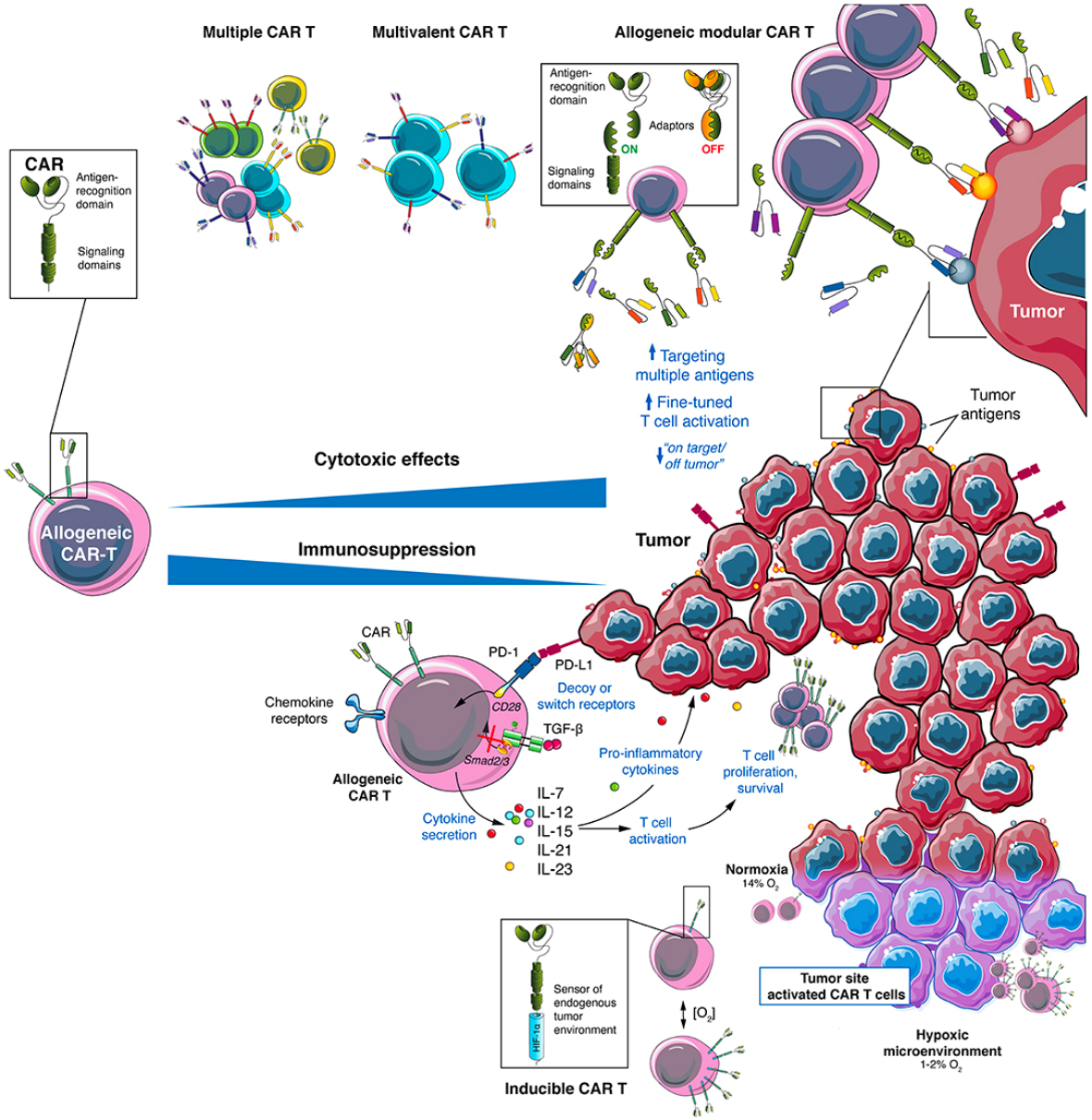
- inhibit NK elimination by increasing expression of Siglec ligands of HLA-E and G variants.

- Anti CD38 Ab treatment

- To protect patients from GVHD, allogeneic CAR T cells can be engineered to lose TCR expression.

Bedoya et al. Frontiers in Immunology 2021

Off the shelf allo CAR T cells



Several strategies to engineer allogeneic CAR T cells can be used:

- secretion of pro-inflammatory cytokines (such as IL-7, IL-12, IL-15, IL-21, or IL-23)

- expression of decoy or switch receptors (to change immunosuppressive signals into activating ones)

- expression of chemokine receptors (to direct CAR T cells to the tumor site)

- generation of locally activated CAR T cells (such as hypoxia-inducible CAR T cells).

Bedoya et al. Frontiers in Immunology 2021

Expansion of CAR T into non-malignant disease

Erlangen Experience (Andreas Mackensen et al. Nature Medicine 2022)

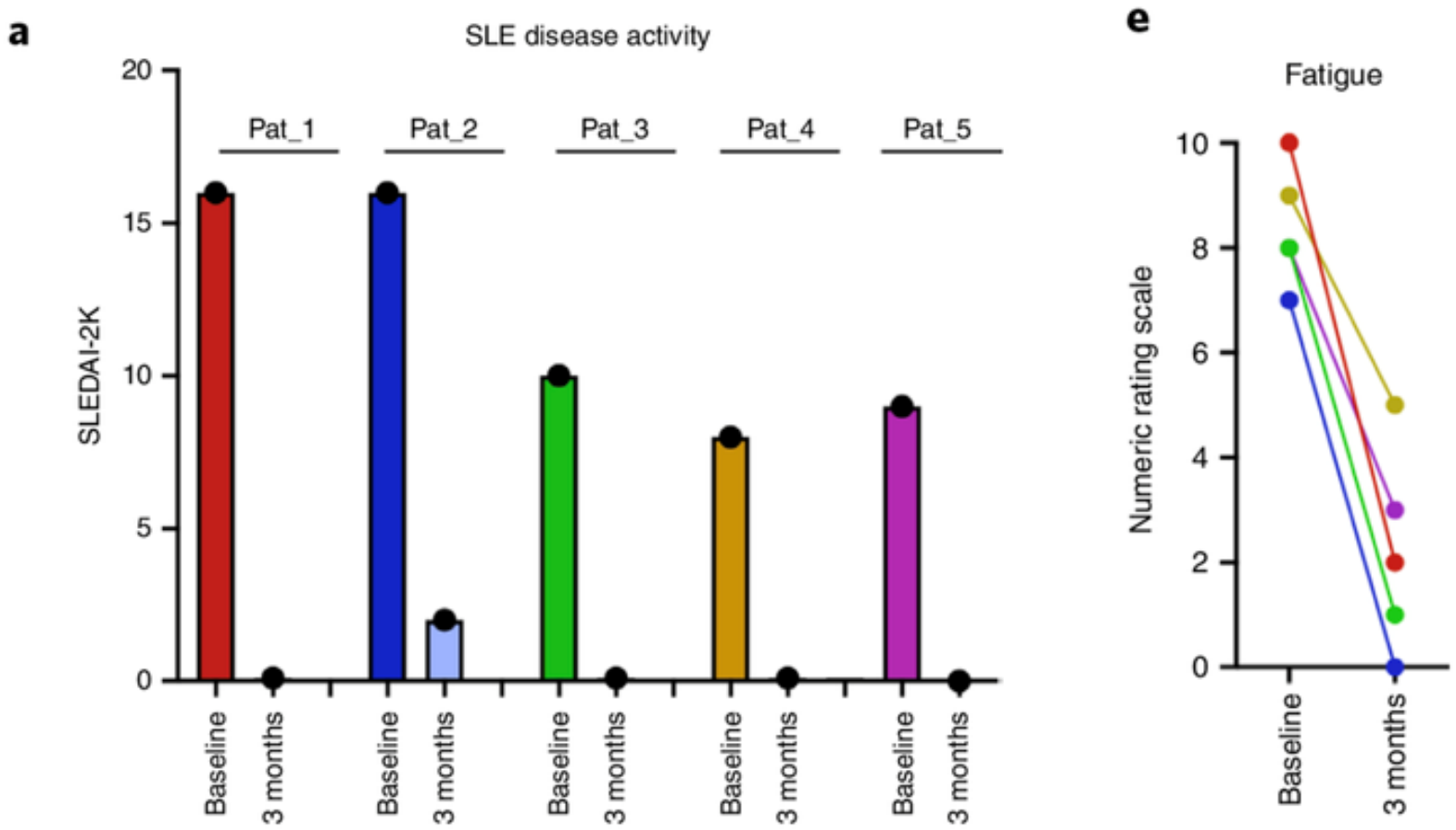
- 5 patients all with active systemic lupus erythematosus (SLE)

- Inclusion:
- Diagnosis of SLE according to the EULAR/ACR 2019 criteria
- Signs of active organ involvement, including kidney involvement (WHO III of IV)
- Failure to respond to multiple immunomodulatory therapies including
  - Repeated pulsed glucocorticoids, hydroxychloroquine
  - Cyclophosphamide
  - Belimumab
  - MMF
  - Rituximab

Expansion of CAR T into non-malignant disease

Erlangen Experience (Andreas Mackensen et al. Nature Medicine 2022)

- Treatment with Miltenyi anti-CD19 CAR T, 4-1BB co-stimulatory domain, 1E10E6/kg BW

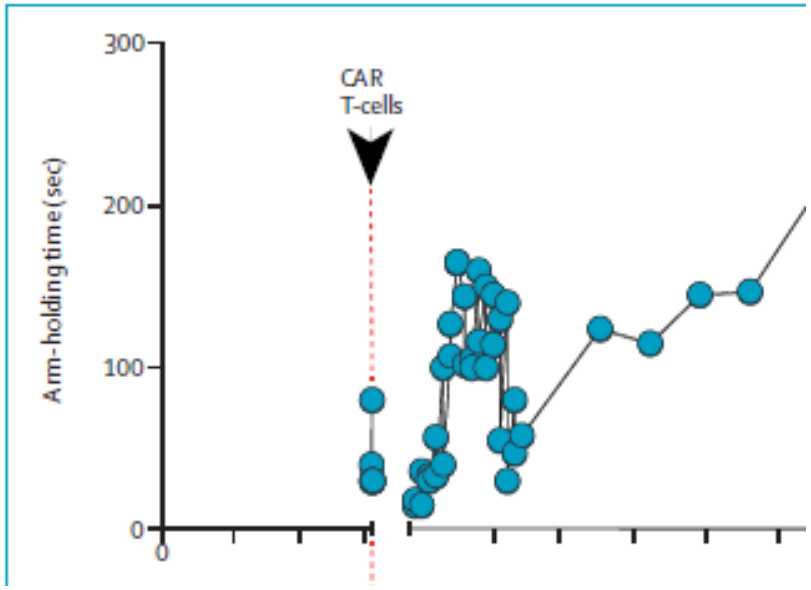


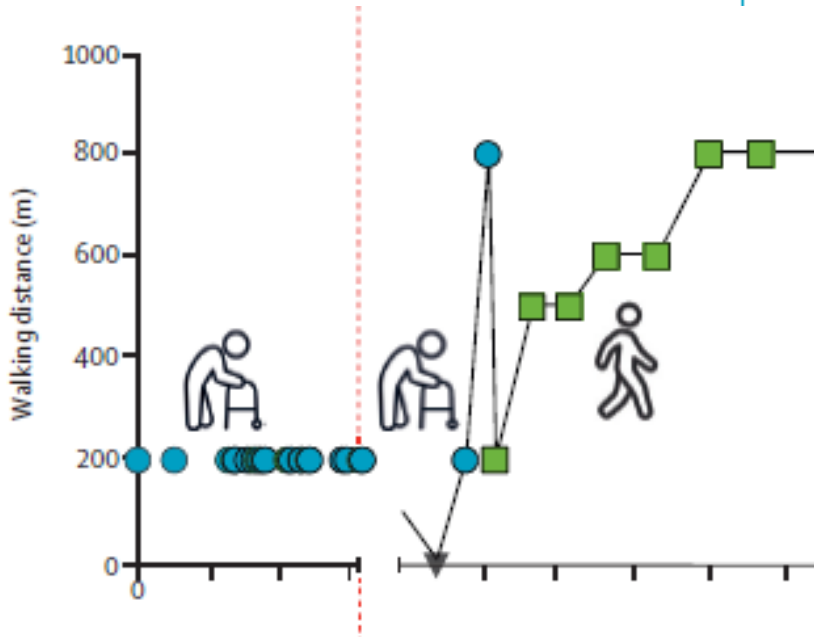
- other refractory autoimmune diseases?

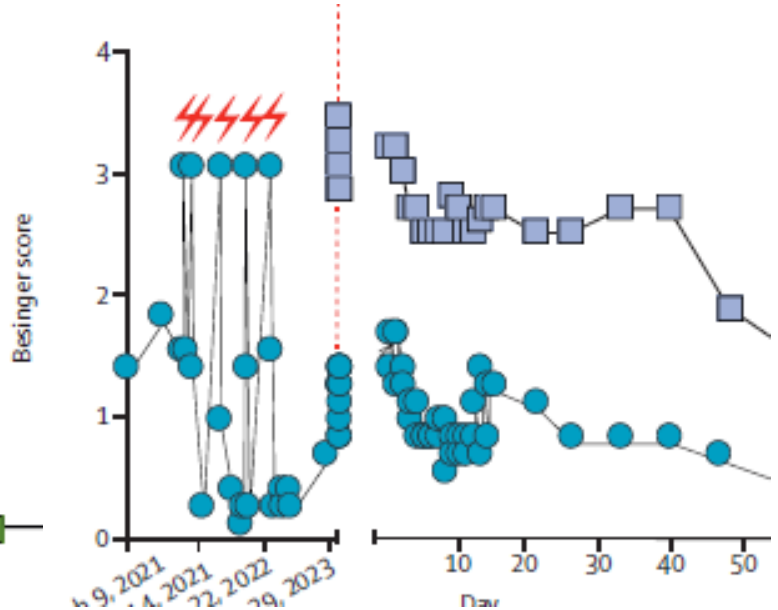
Expansion of CAR T into non-malignant disease

Mougiakakos D, Lancet Oncology 2023

- Treatment with Kyverna anti-CD19 CAR T, CD28 co-stimulatory domain, 1E10E8 cells







Advances in CAR T for solid tumors: Opportunities

- Tumor Associated Antigens (TAAs):
  - Mucin-1 (tumor antigen related to tumorigenesis, invasion and metastasis)
  - CD276 (cell surface expressed immune checkpoint molecule, immune-inhibitory on T cell and NK cell activity)
  - HER2 (transmembrane glycoprotein)
  - EGFR
  - CEA
  - Mesothelin
  - GD2 (expressed by a variety of embryonal cancers, including brain cancers, but barely on normal cells
  - EpCAM (overexpressed in carcinomas, incl. colorectal, gastric, pancreatic, endometrial cancers)

Yan T et al., Exp Hematol Oncol, 2023

Table 1. Several targeted antigens utilized in CAR T cell therapy for solid tumors in clinical trials.

Type of Cancer	Targeted Antigens
Glioblastoma	HER2, IL13Ra2, EGFRviii
Neuroblastoma	GD2, GPC2, CD171
Lung cancer	MSLN, EGFR, FAP, CEA, PSMA, MUC1, ROR1
Mesothelioma	MSLN, FAP
Breast cancer	c-Met, MSLN, HER2, GD2, CD44v6, MUC1, EpCAM
Gastric cancer	Claudin18.2, HER2, MSLN
Hepatocellular carcinoma	GPC-3, MSLN
Pancreatic cancer	MSLN, EGFR, CEA, HER2, PSCA, CLDN18.2, CD133
Renal cell carcinoma	CAIX, AXL, ROR2, EGFR, MSLN
Colorectal cancer	TAG-72, CEA, NK2GD, GUCY2C, DCLK1
Ovarian cancer	FRa, MSLN, MUC1, NKG2D, HER2, CD276, TAG72, MUC16, 5T4
Prostate cancer	PSMA

Ma et al., Cur Issues Mol Biol 2023

Advances in CAR T for solid tumors: Challenges

- on target, off – tumor toxicity, e.g. HER2 or EGFR TAAs

- limited number of tumor neoantigens, e.g. BRAF, KRAS, TP53

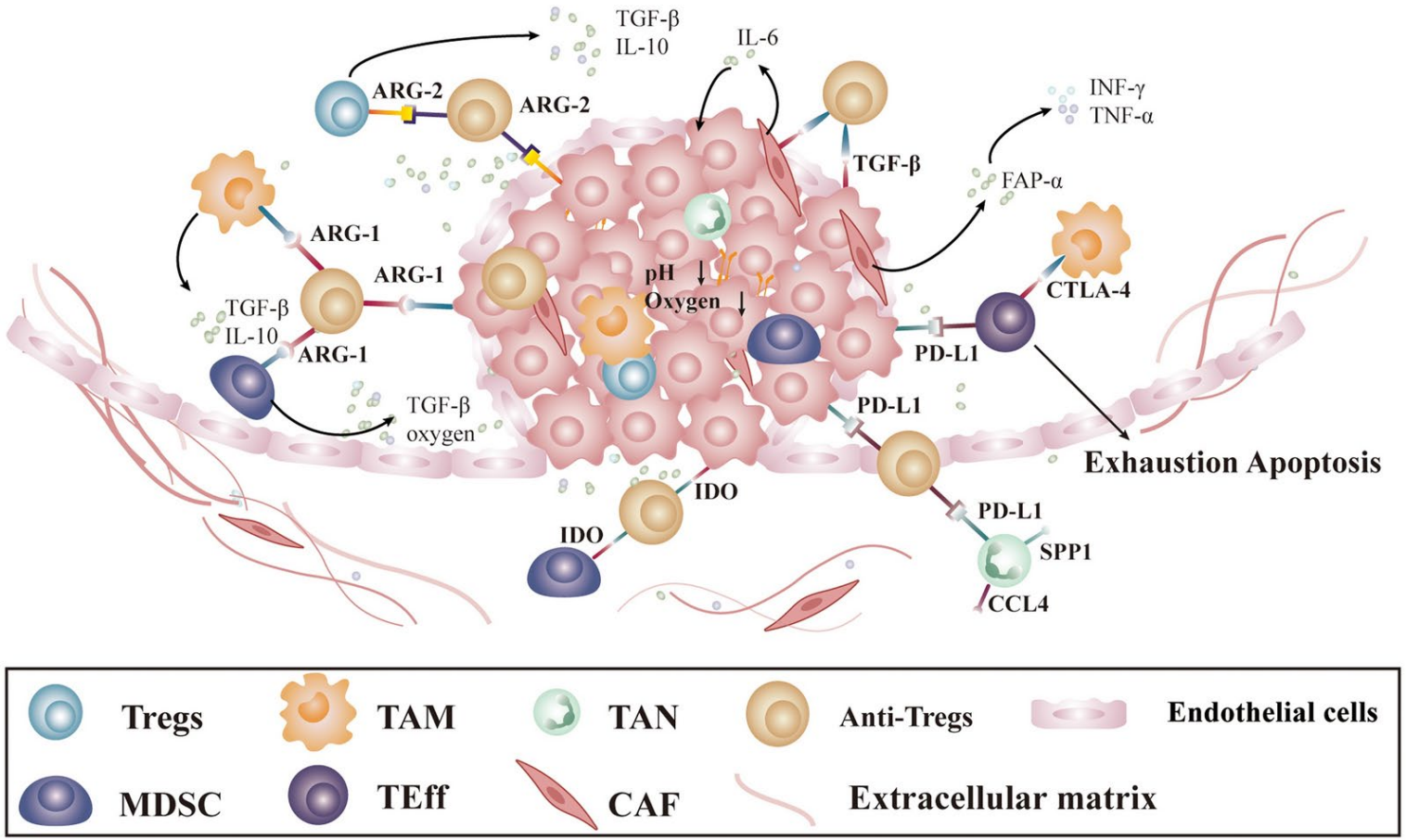
- tumor immunosuppressive microenvironment

- inefficient infiltration of CAR T in solid tumors
    - e.g. due decreased chemokine gradients, dense fibrotic stroma
        - possible intervention: PARP inhibitors via cGAS-STING pathway activation

- chronic antigen stimulation leads to CAR T-cell exhaustion
        - possible intervention: perturbation of INO80 and BAF chromatin remodeling complexes

Yan T et al., Exp Hematol Oncol, 2023

Advances in CAR T for solid tumors: Tumor microenvironment antigens (TMAs): challenge and opportunity



Yan T et al., Exp Hematol Oncol, 2023

Advances in CAR T for solid tumors: Opportunities

- Dual CAR and tandem CAR

- targeting TMAs

- nanobodies (VHH antibodies), derived from variable domain of a heavy chain antibody, can bind to antigen recognition sites

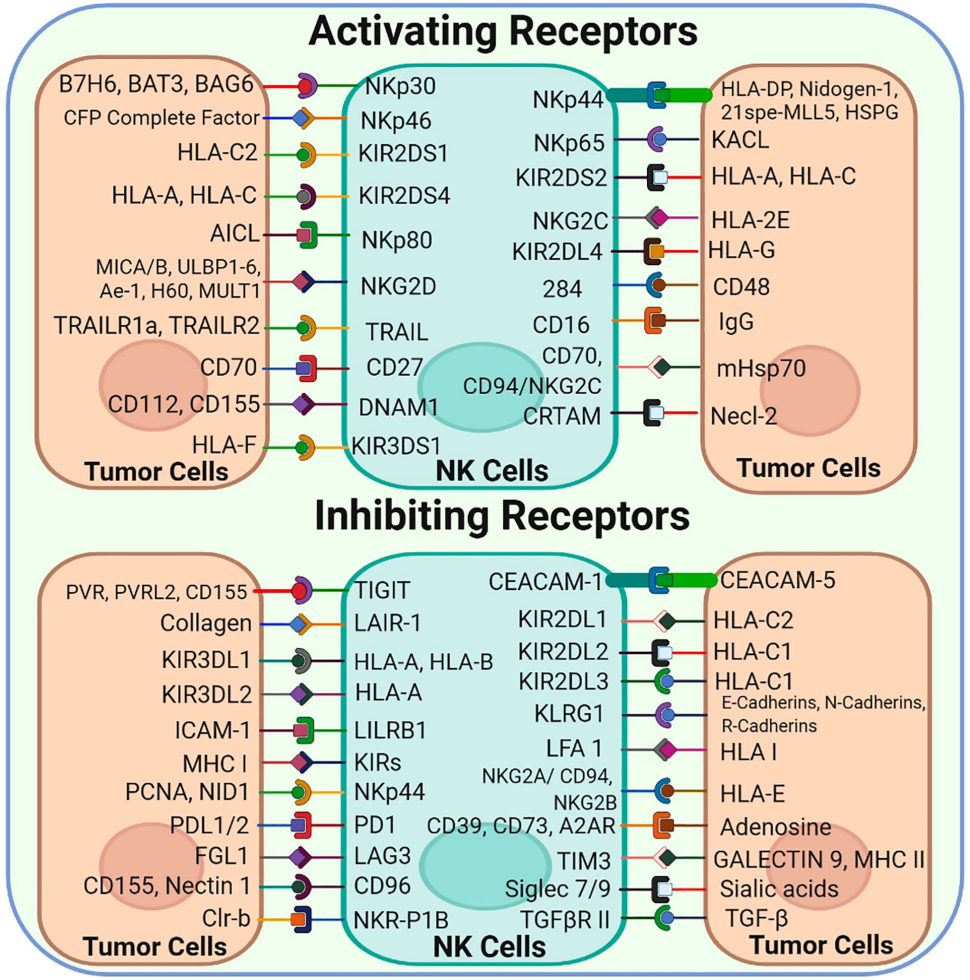
Yan T et al., Exp Hematol Oncol, 2023

Gamma / delta CAR T (solid tumors)

- represent 1 to 10% of total $CD3_+$ T-cells
- express a combination of either of 7 different Vγ TCR chains (Vγ2, 3, 4, 5, 8, 9, and 11), paired with either of 4 Vδ (Vδ1, 2, 3, and 5) chains.
- γδ T-cells are considered to bridge the innate and adaptive immune systems
- Activated γδ T-cells display strong cytotoxic activity through the release of granzyme B and perforin, by membrane bound TRAIL and Fas (CD95) ligands or production of IFNγ or TNFα to amplify the immune response
- High γδ T-cell frequency in tumor infiltrates from cancer patients correlates with better clinical outcome in different malignancies
- γδ T-cells were identified as the prognostically most favorable immune cell subset in tumor infiltrates from 18,000 tumors across 39 malignancies

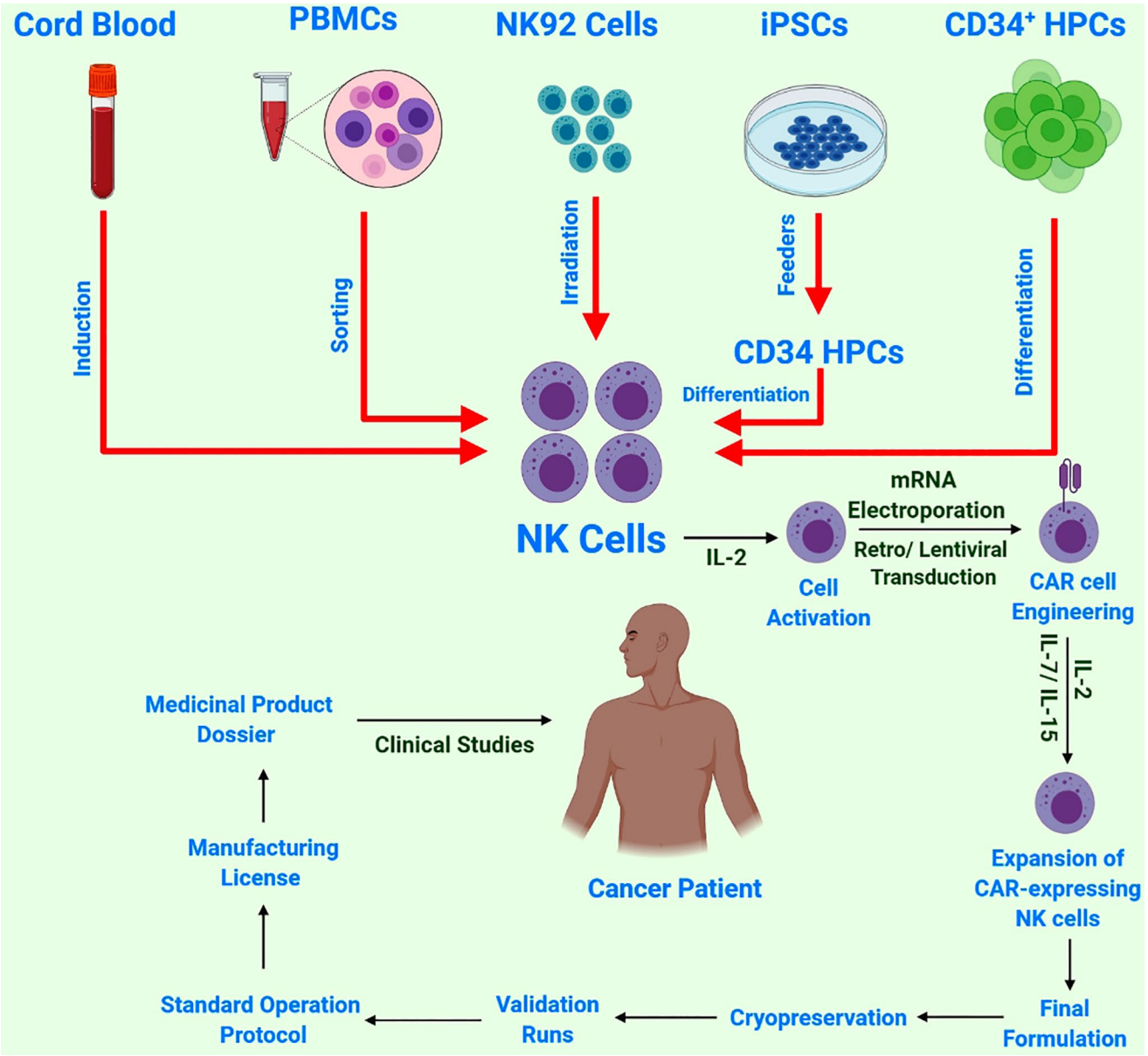
Reviewed in: Saura-Esteller et al., Front Immunol 2022

NK-CARs



Khawar et al. Frontiers in Immunology 2021

NK-CARs



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NK-CARs

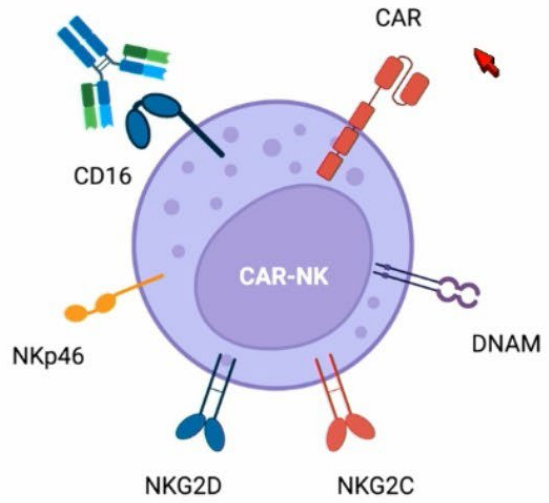
NK cells for allogeneic CAR therapy

Advantages:

- Allogeneic: no GVHD --> off the shelf, lower cost
- Killing: CAR mediated + innate receptors
- Antibody-dependent cellular cytotoxicity (ADCC) through binding of CD16 on NK cells to antibody-bound target cells
- Safety: no CRS, no ICANS

Disadvantages:

- Limited lifespan in the absence of cytokine support
- Unclear best starting population for manufacturing



Rezvani ASH 2023 ED Thomas Lecture

NK-CARs

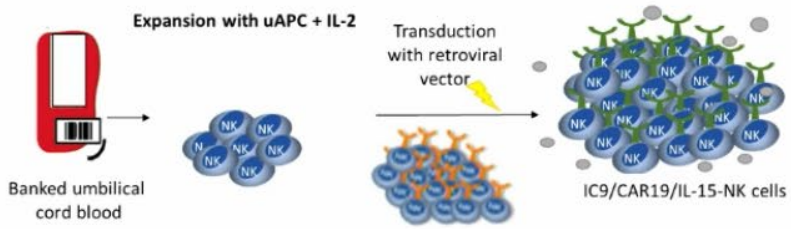
Properties	CAR-T	CAR-NK
Low risk of GVHD		✓
High tumor-killing potential	✓	✓✓
Low risk of Cytokine release syndrome		✓
High graft-versus-tumor (GVT) potential		✓
Low cost off-the-shelf cancer immunotherapy	✓	✓✓
Sources of harvestation	✓	✓✓

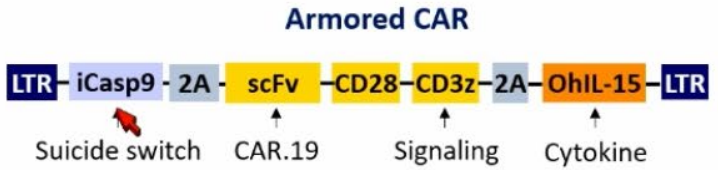
A comparison of CAR-T and CAR-NK immunotherapy: CAR-NK cell therapies are becoming increasingly popular due to several advantageous features such as low safety concerns, low costs, and higher tumor potential.

Khawar et al. Frontiers in Immunology 2021

NK-CARs

First in-human trial of CAR19/IL-15 CB-NK cells in lymphoid malignancies



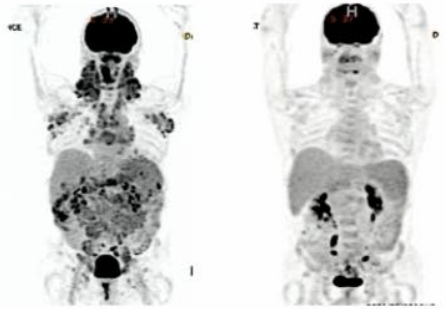


Generously provided by Pietro Dotti, MD

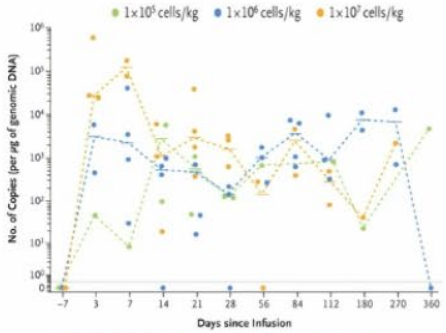
Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D., Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D., Lucila Nassif Kerbauy, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D., Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D., Ana Nunez Cortes, M.D., Kai Cao, M.D., May Daher, M.D., Chitra Hosing, M.D., Evan N. Cohen, Ph.D., Partow Kebriaei, M.D., Rohtesh Mehta, M.D., Sattva Neelapu, M.D., Yago Nieto, M.D., Ph.D., Michael Wang, M.D., William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D., Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.

N Engl J Med. 2020 Feb 6; 382(6): 545–553.



7/11 CR, no CRS, no neurotoxicity, and no GvHD



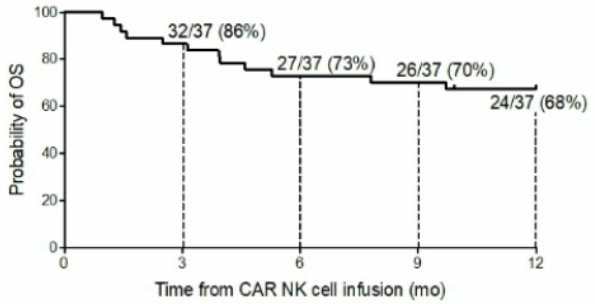
CAR-NK cells are detectable up to 12 months post-infusion

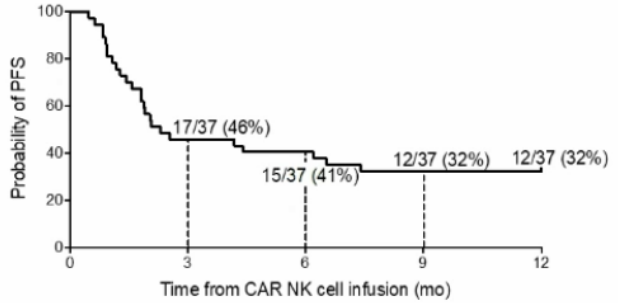
Liu et al. & Rezvani *N Engl J Med*, 2020

Rezvani ASH 2023 ED Thomas Lecture

NK-CARs

CAR19/IL-15 NK cell therapy results in durable responses in patients with lymphoid malignancies-final trial data (n=37)



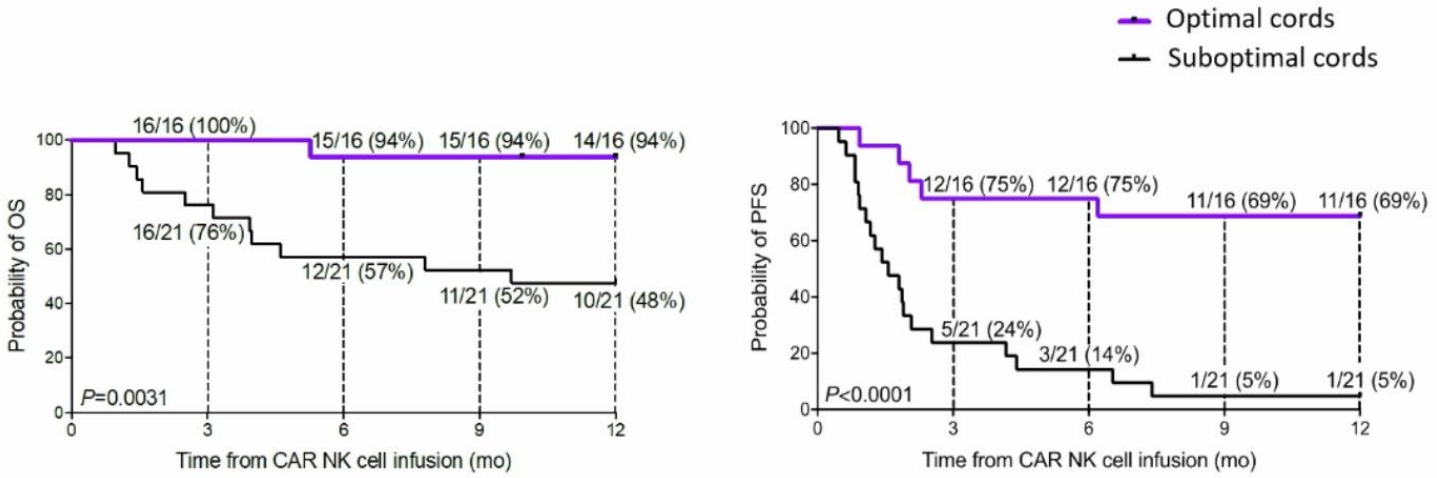


Provisionally accepted, *Nature Medicine 2023*

Rezvani ASH 2023 Ham Wasserman Lecture

NK-CARs

Patients who received CAR19/IL-15 from optimal cords had superior response when compared to those obtained from suboptimal cords



Optimal cords: Time to freezing ≤24hr; NRBC ≤8E7

Suboptimal cords: Time to freezing >24hr; NRBC >8E7

Provisionally accepted, *Nature Medicine 2023*

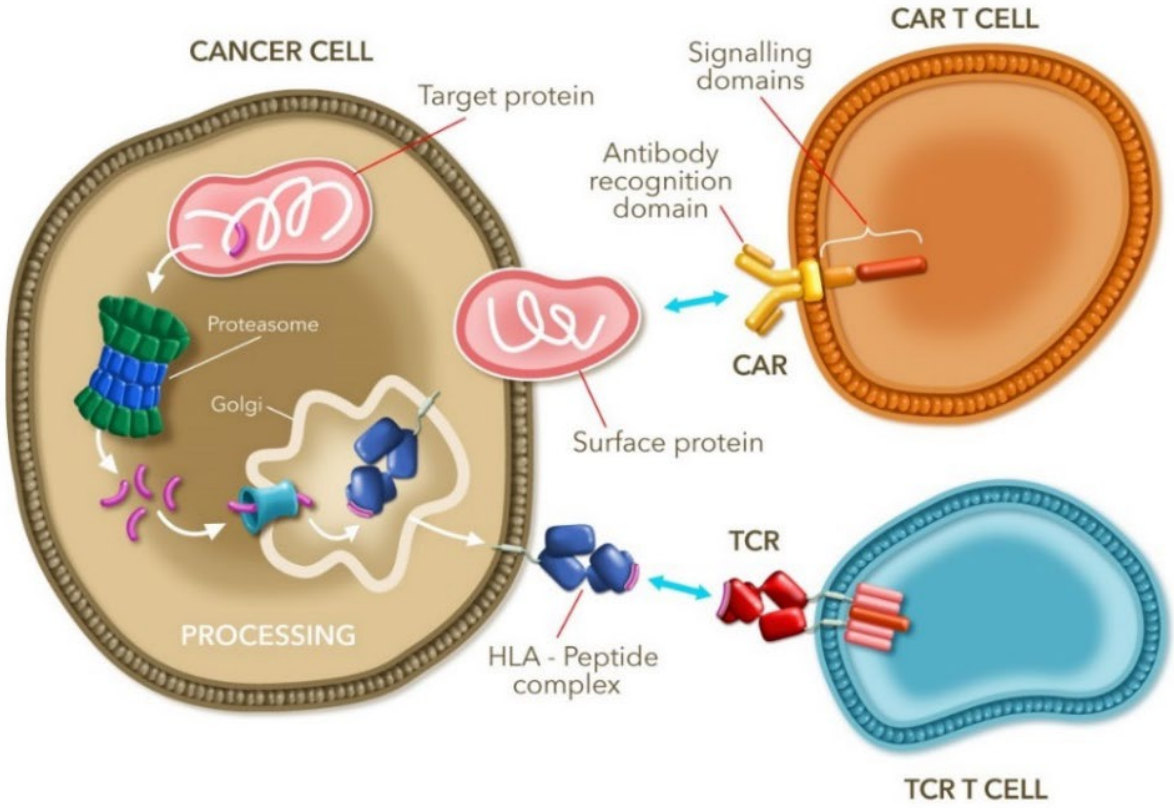
Rezvani ASH 2023 Ham Wasserman Lecture

T cell receptor T (TCR-T)

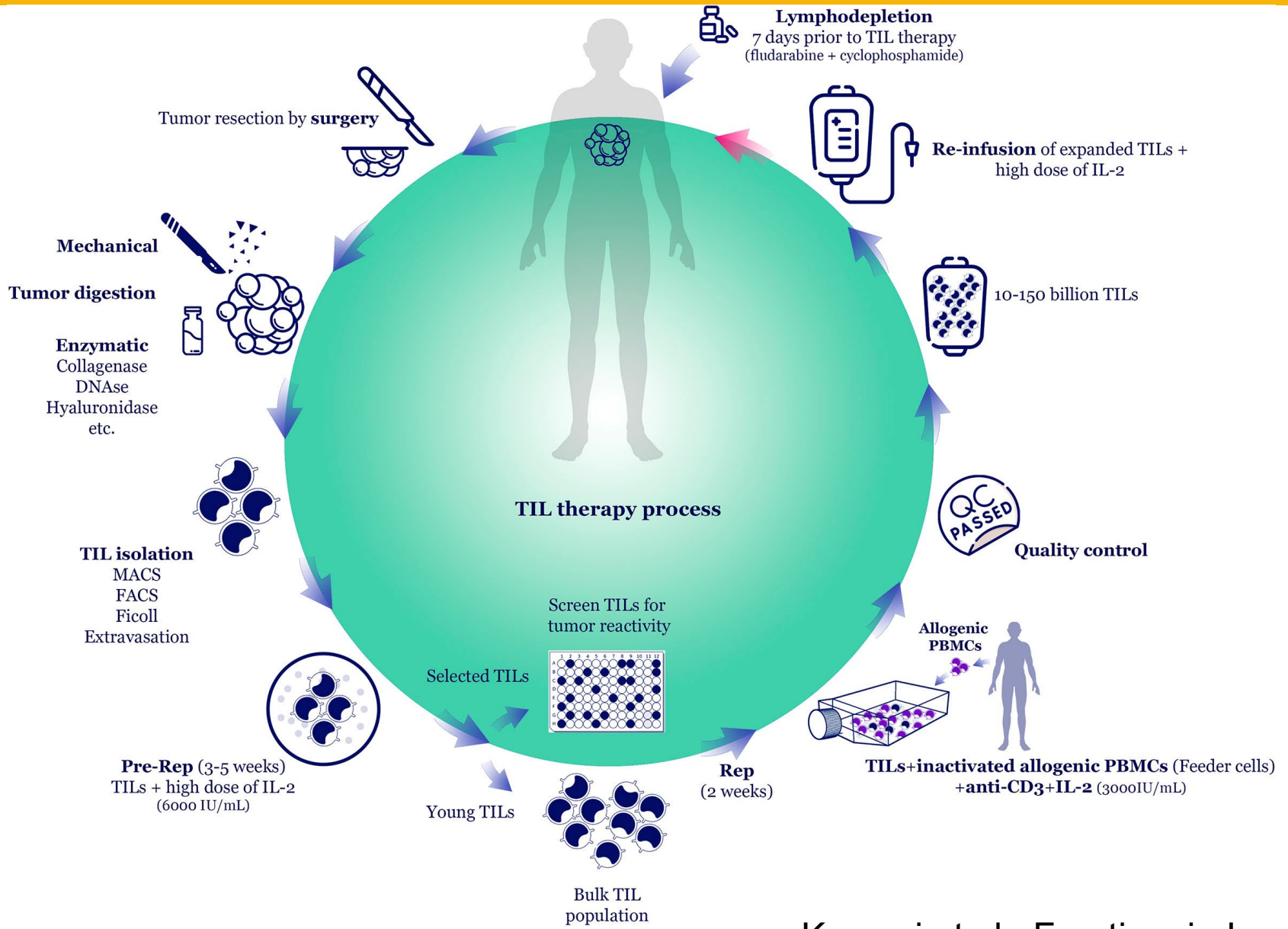
T cell receptor:

- targets are intra- and extracellular proteins (mHags, viral proteins, tumor-overexpressed proteins, e.g. NY-ESO-1
- Usually, peptides expressed in the context of MHC class I
- Involves regular T cell receptor / MHC I interactions but requires MHC-1 / HLA matching
- TCR-T potentially efficacious in solid tumors

TCR-T


CANCER CELL
Target protein
Proteasome
Golgi
PROCESSING
CAR T CELL
Signalling domains
Antibody recognition domain
CAR
Surface protein
TCR
HLA - Peptide complex
TCR T CELL

Tumor infiltrating lymphocytes (TILs)



Kazemi et al., Frontiers in Immunology 2022

Tumor infiltrating lymphocytes (TILs)

- Needs cellular therapy expertise due to IL-2 treatment and cytokine release

- metastatic melanoma (about to be FDA approved)
- Cervical cancer
- Ovarian cancer
- Breast cancer

Kazemi et al., Frontiers in Immunology 2022

Thank you !