CAR T-cell Therapy: Patient Selection and Preparation

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Disclosures

- Consulting for Kite/Gilead, Novartis, BMS/Celgene, Caribou Bio, AztraZeneca, ADC Therapeutics, Abbvie, Miltenyi, Galapagos, Lyell, Autolus, Umoja, Kyverna, GenmAb, Genentech, ADC Therapeutics

- Research funding from Kite/Gilead

Factors Associated with Outcome: Defining Patient Selection

Predictors of Response and Toxicity

PATIENT

T-CELLS

TUMOR

PRE-TREATMENT

POST-TREATMENT

<u>Improved Response</u>

- Low tumor burden, low LDH
- Low pretreatment inflammatory markers
- Absence of medical comorbidities
- Lack of need for bridging therapy

- Proportion of CCR7+ and other early memory T-cells in the CAR product
- Faster doubling time *in vitro*
- Higher CAR T-cell peak to tumor burden ratio

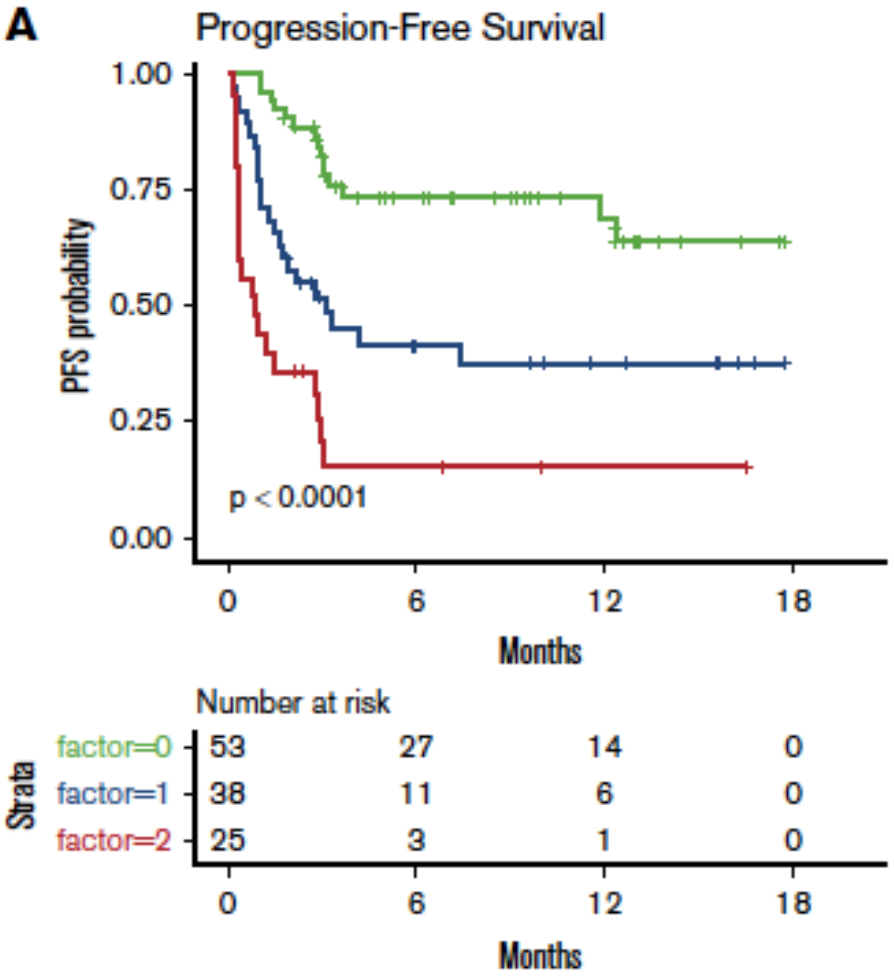
- Genetic/genomic factors (ie CD58 mutations in LBCL)
- Low tumor MDSCs
- High TILs
- Absence of MYC overexpression

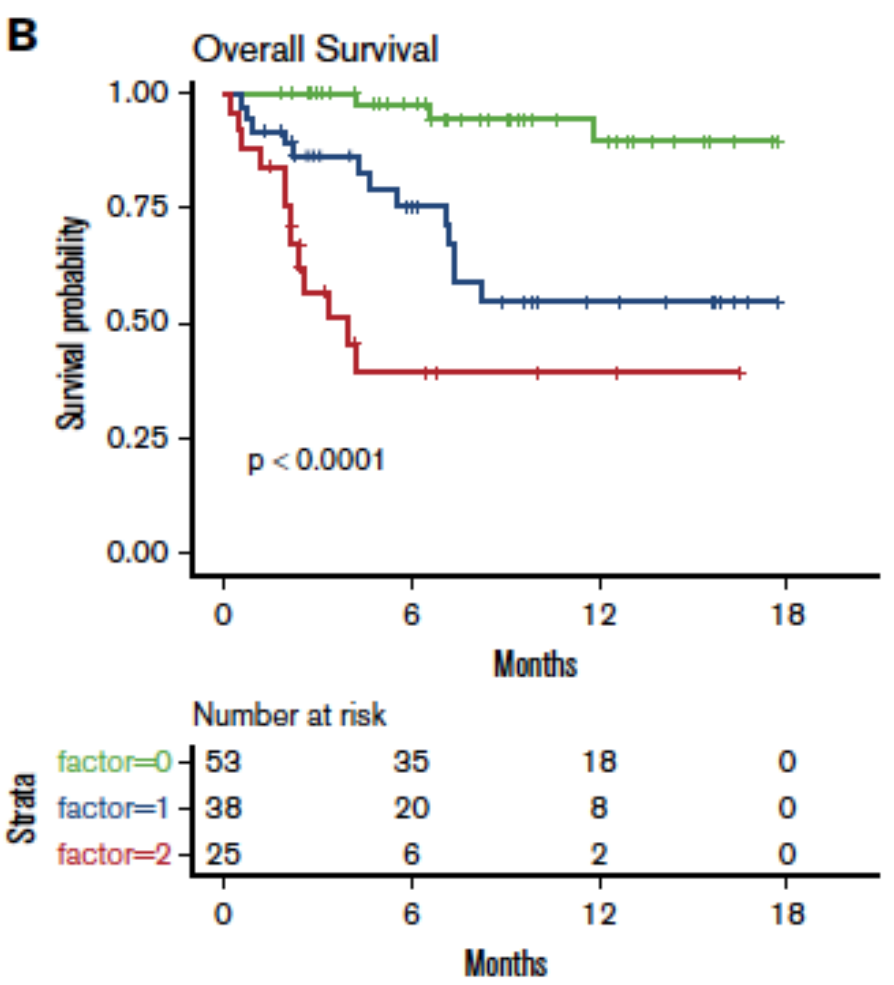
<u>Increased Toxicity</u>

- High tumor burden, pretreatment LDH
- High pretreatment inflammatory markers
- ? High pretreatment monocyte levels

- High peak CAR T-cell levels
- High peak cytokine levels
- Markers of DIC (including fibrinogen levels)
- Early CRS

TMTV and EN Disease

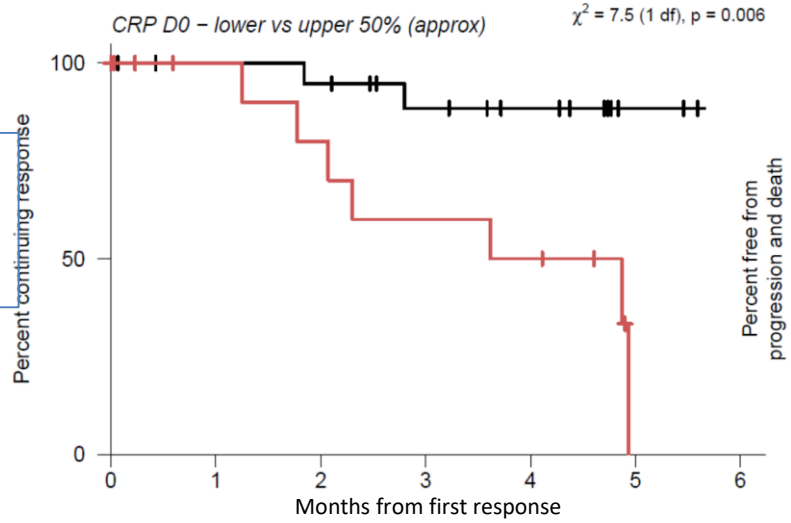




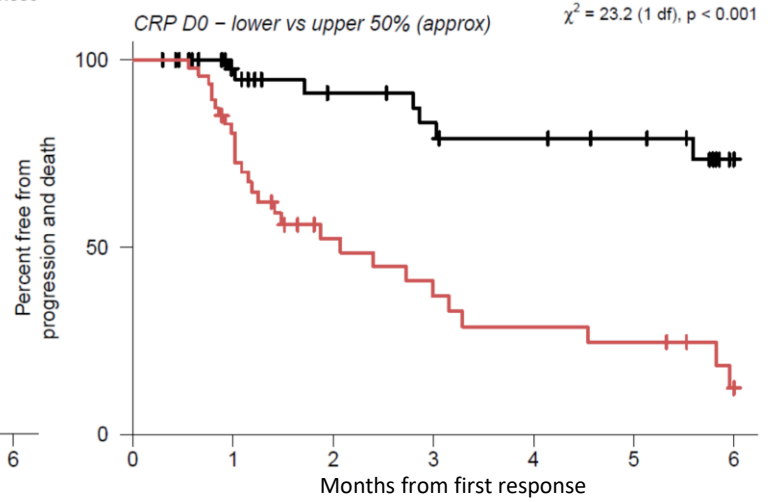
Vercellino et al Blood Adv 2

Day 0 CRP and Peak Ferritin are Associated with Outcome

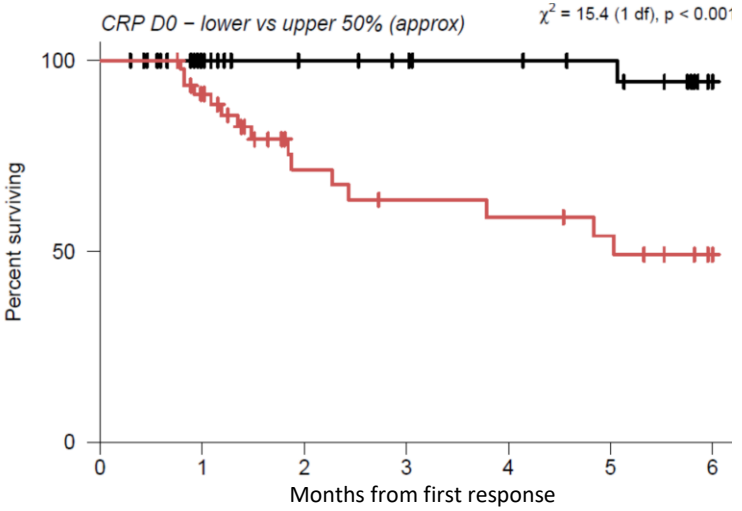
Day 0 CRP:
<30
>30



Duration of Response

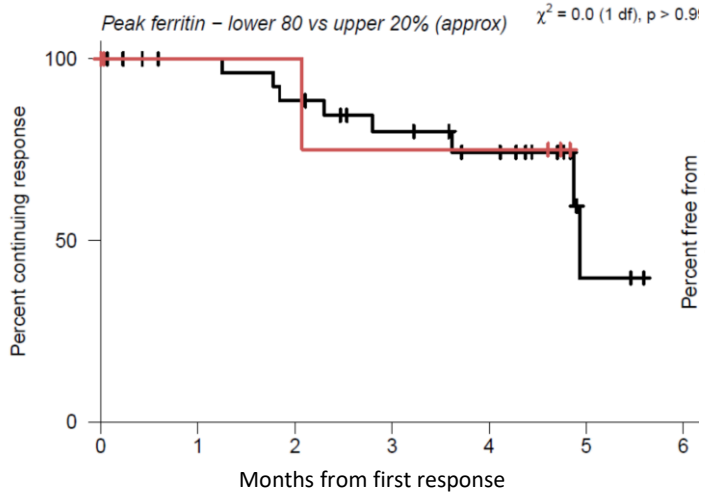


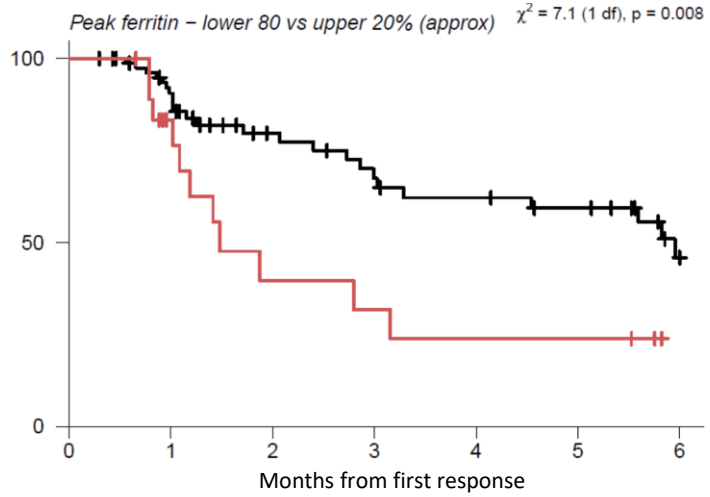
Progression-free Survival

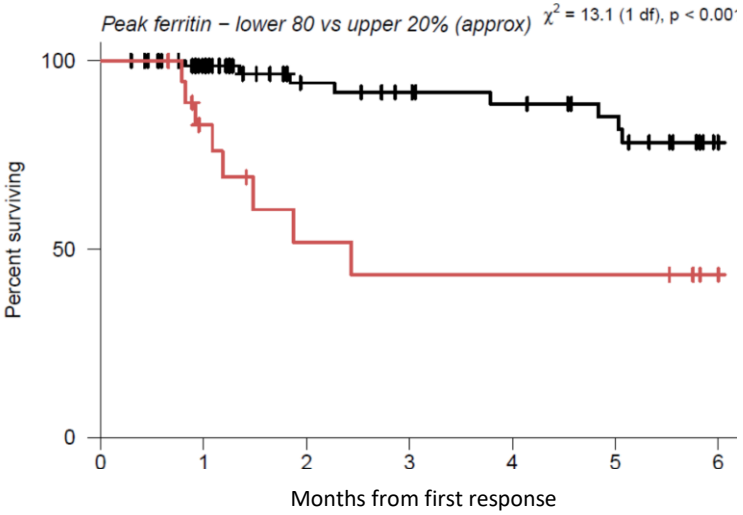


Overall Survival

Peak Ferritin:
<5000
>5000





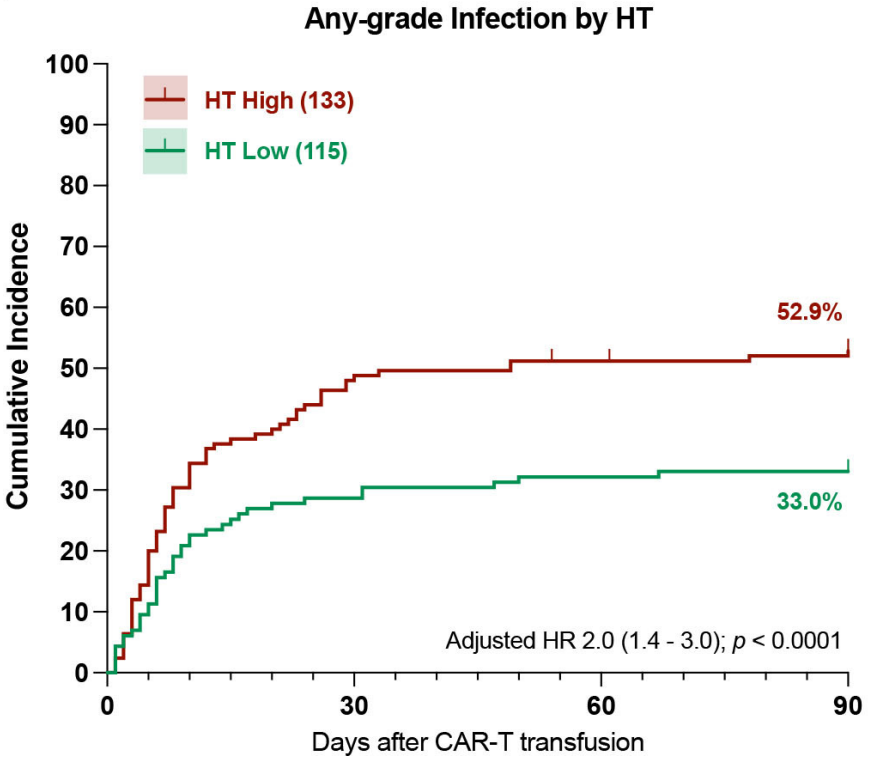


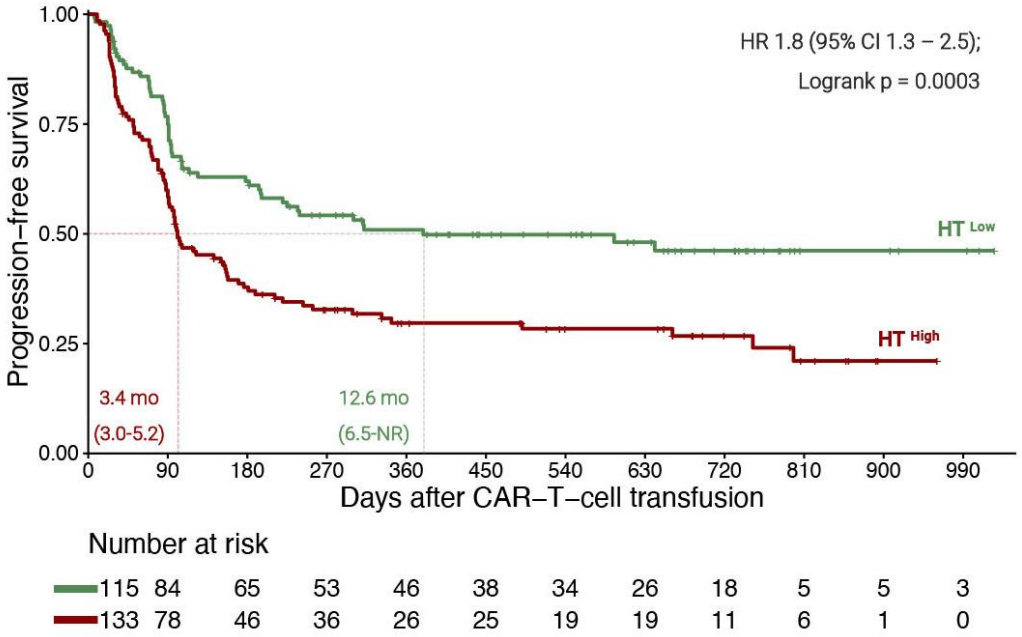
CAR HEMATOX Score

1-2 points for each on 1st day of LD chemo:

- ANC <1200
- Hgb <9
- Plt <75 or 75-175
- CRP >3
- Ferritin 650-2000 or >2000

High HT score 2+ points





Rejeski K, et al. J Immunotherapy Cancer. 2021;10:e004475.

Case : DLBCL

A man in his 60s was diagnosed with a triple hit lymphoma.

He received da-REPOCH with residual, progressive disease at the end of treatment

He received RICE with no response

Had T-cells collected for CAR T-cells followed by the development of gangrenous necrosis of the gluteus requiring extensive surgery and debridement, post-surgery requiring extensive physical therapy

Was bridged with a cycle of polatuzumab/rituximab while awaiting PS improvement to proceed with CAR T-cell infusion

※紹介した症例は臨床症例の一部を紹介したもので、全ての症例が同様な結果を示すわけではありません。
※高齢者では一般に生理機能が低下しているので、患者の状態を観察しながら慎重に投与すること

自験例

Case : DLBCL

A man in his 60s with primary refractory triple hit lymphoma with CAR T-cell treatment delayed 2/2 gangrene s/p surgery;

Received axi-cel followed by placement of percutaneous nephrostomy tubes bilaterally for obstructive uropathy

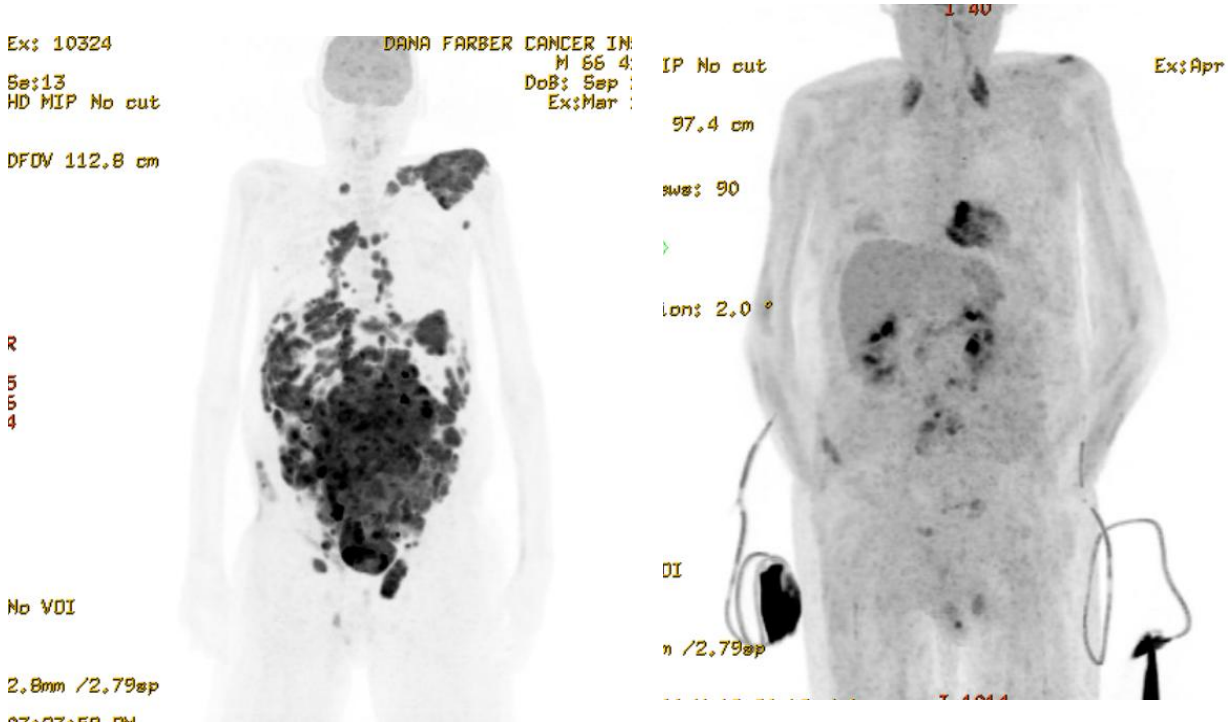
- Grade 2 CRS treated with toci/dex
- Prolonged grade 3 ICANS treated with prolonged course of dex

自験例

Case : DLBCL

A man in his 60s with primary refractory triple hit lymphoma with CAR T-cell treatment delayed 2/2 gangrene s/p surgery;. Course c/b grade 2 CRS and prolonged grade 3 ICANS and obstructive uropathy s/p bilateral PCNs

At the 4-year follow-up, disease control and management of adverse events have been maintained, and the patient continues to carry out daily activities



自験例

Patient Selection

There are a number of predictors of poor PFS but CAR outperforms other available therapies for each of these patients.

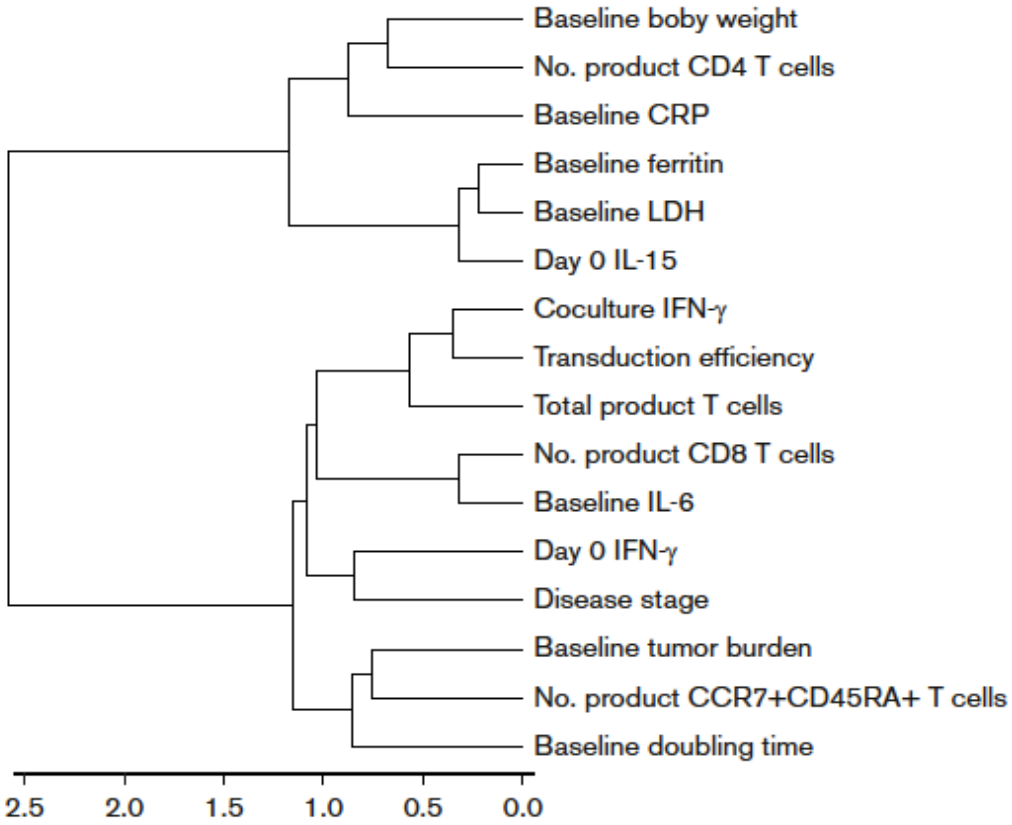
Objective, then, should be to optimize patients prior to CAR (short-term) and to understand and respond to mechanisms of resistance (long-term)

In the meantime, early referral of all eligible patients is essential!

- **Referral should be one line of therapy earlier than CAR is indicated, which is challenging in 2L**
- **Given this, high-risk newly diagnosed DLBCL (IPI 4-5, HGBL, DHL/THL) referrals at diagnosis so they are established if they should need 2L CAR is advantageous**

Mechanisms of Resistance: How to Maximize Patient Outcomes

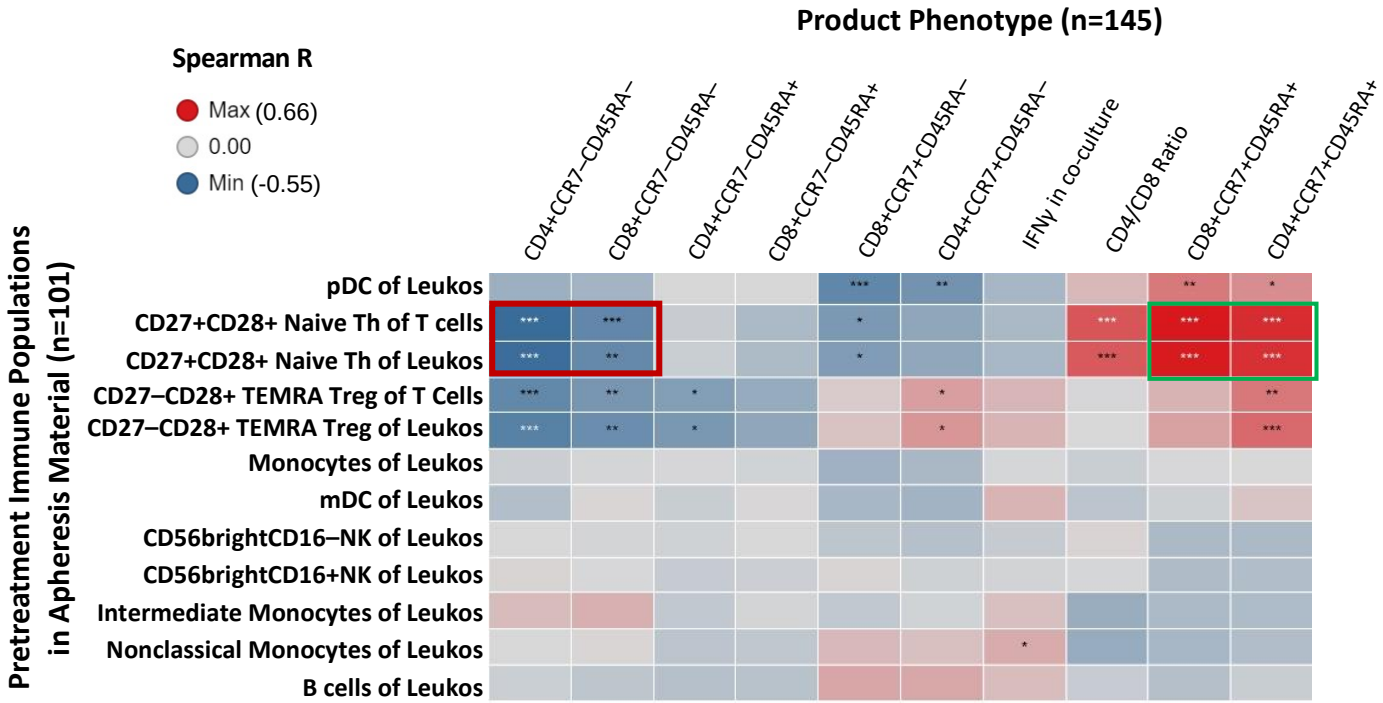
Tumor and Product Attributes Correlate with Durable Response to Axi-cel on ZUMA-1



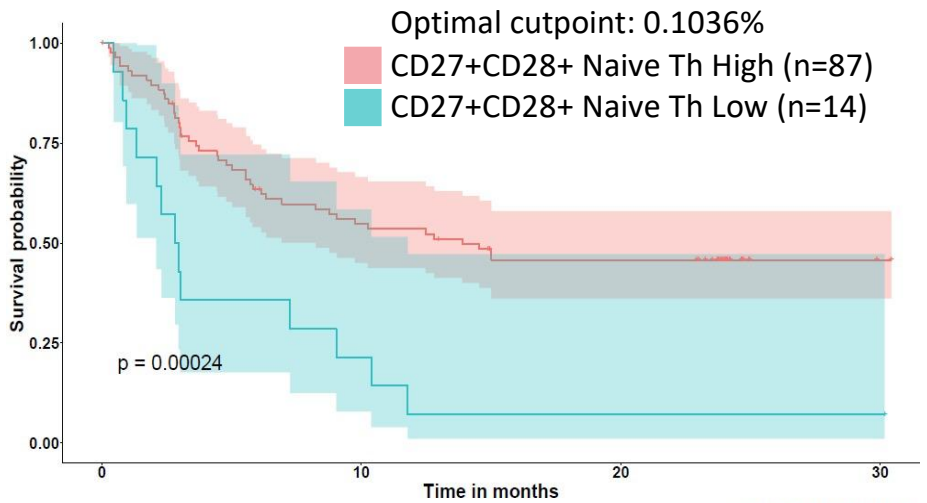
- Tumor burden negatively impacts DOR, but this can be overcome by CAR T-cell expansion
- High levels of pretreatment inflammation and high MDSCs negatively correlate with CAR T-cell expansion
- Product attributes including high CD8+ cells and naïve T-cells overcome the negative influence of high tumor burden on DOR
  - These product attributes correlated with high levels of pretreatment CD27+CD28+ naïve T cells and low numbers of intermediate monocytes in the pheresis product as well as a “hot” TME

Locke et al Blood Adv 2020;4:4898; Jain et al Blood 2021;137:2621; Budka & Chou et al AACR 2021

T-cell Phenotype at Pheresis Associate with Prognostic T-cell Phenotypes in the CAR T-cell Product

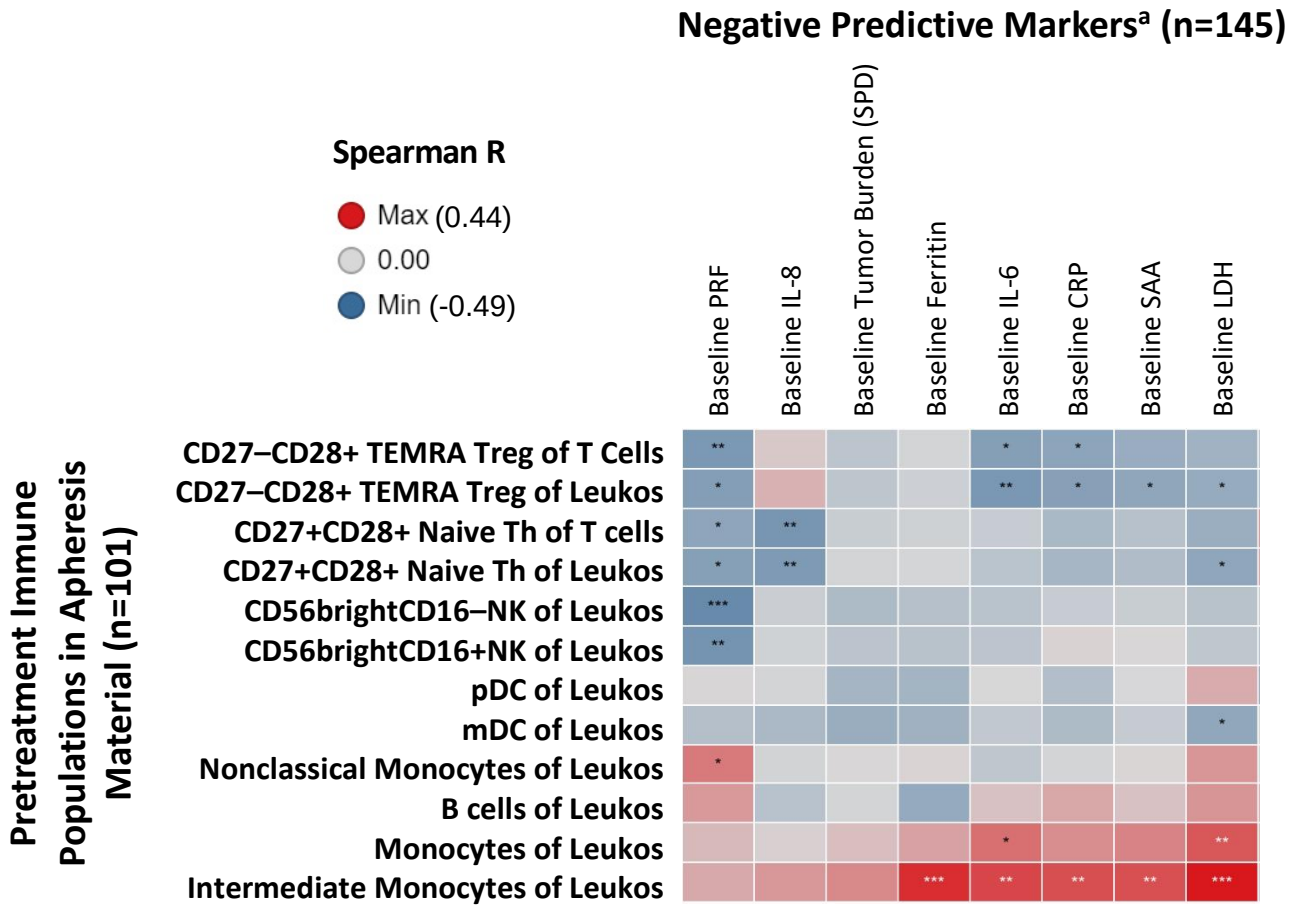


Progression-Free Survival

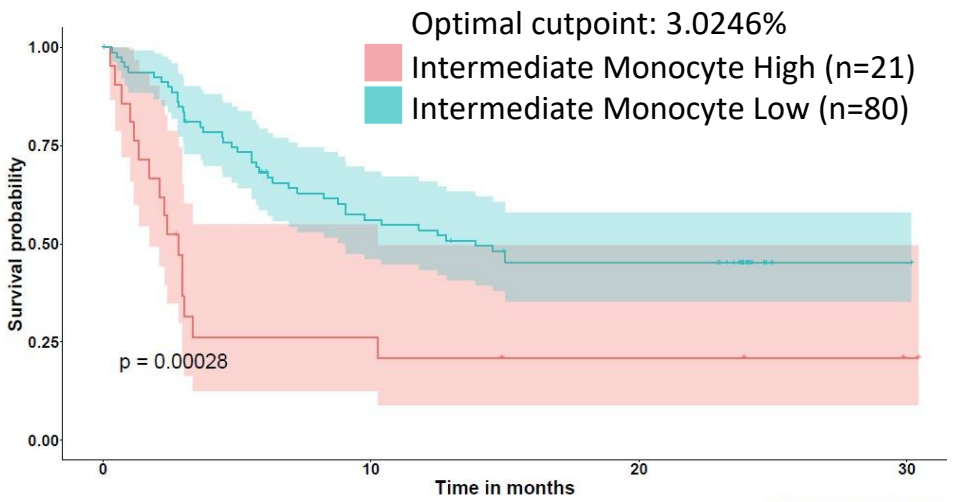


	High (n=87)	Low (n=14)
ORR, n (%)	74 (85)	10 (71)
CR rate, n (%)	52 (60)	7 (50)
Ongoing response, n (%)	36 (41)	2 (14)
Grade ≥3 NEs, n (%)	28 (32)	3 (21)
Grade ≥3 CRS, n (%)	9 (10)	2 (14)
Median CAR peak, cells/µL	42.588	19.836
Median CAR peak/tumor burden, cells/mm^2	0.01105	0.00872

Intermediate Monocytes in Pheresis Product Associate with High LDH and Inflammatory Markers and Worse Outcomes

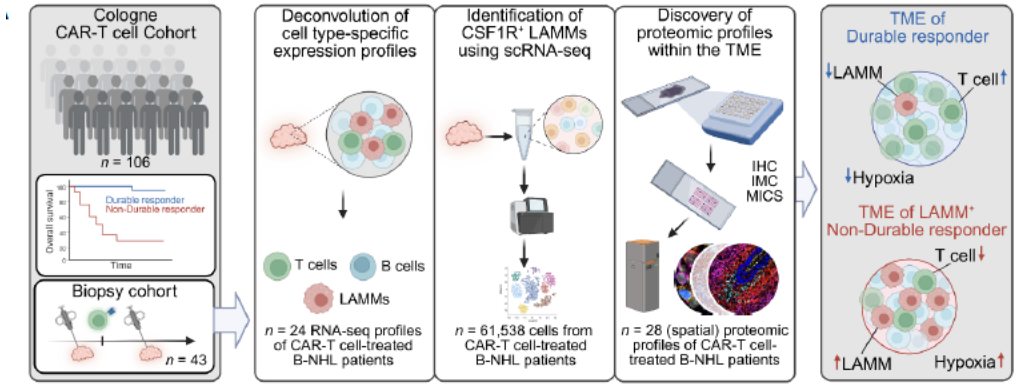


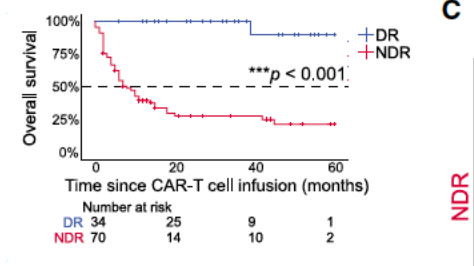
Progression-Free Survival

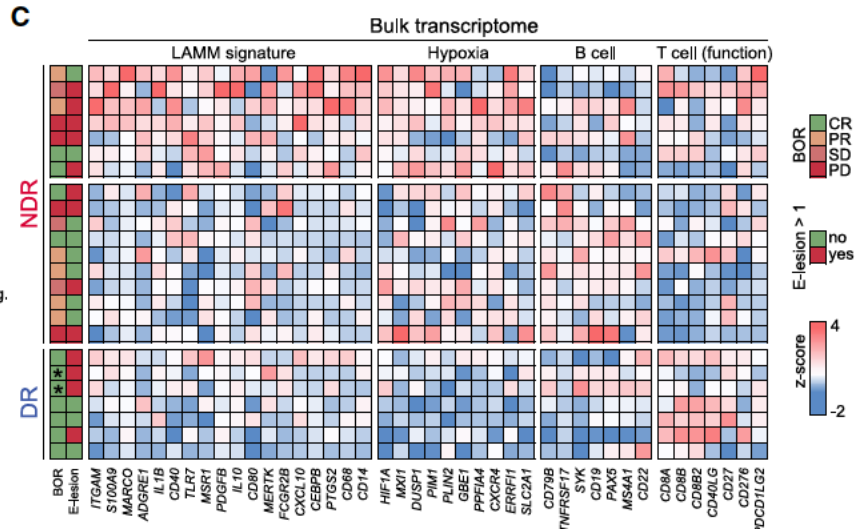


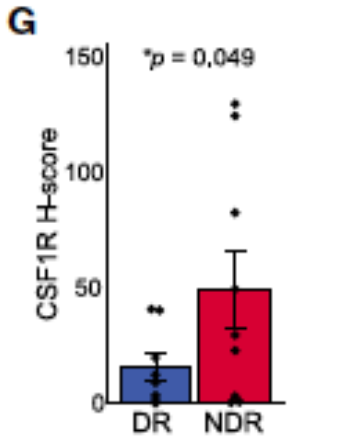
	High (n=21)	Low (n=80)
ORR, n (%)	13 (62)	71 (89)
CR rate, n (%)	5 (24)	54 (68)
Ongoing response, n (%)	4 (19)	34 (43)
Grade ≥3 NEs, n (%)	6 (29)	25 (31)
Grade ≥3 CRS, n (%)	3 (14)	8 (10)
Median CAR peak, cells/µL	48.803	35.273
Median CAR peak/tumor burden, cells/mm^2	0.00757	0.01202

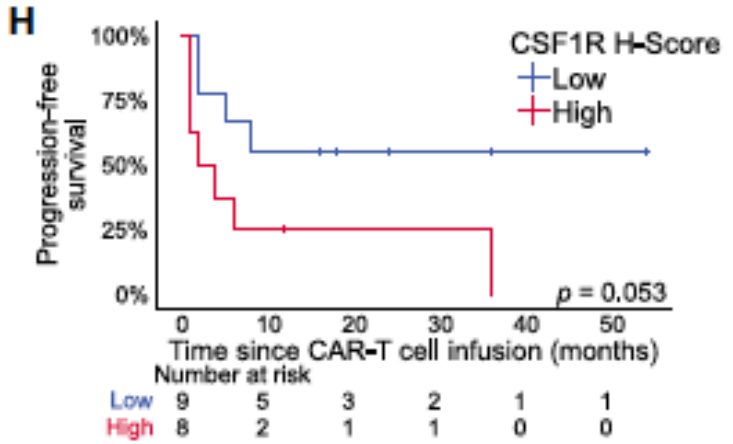
Increased CSF1R+ LAMMs Associate with Poor Outcome after CAR

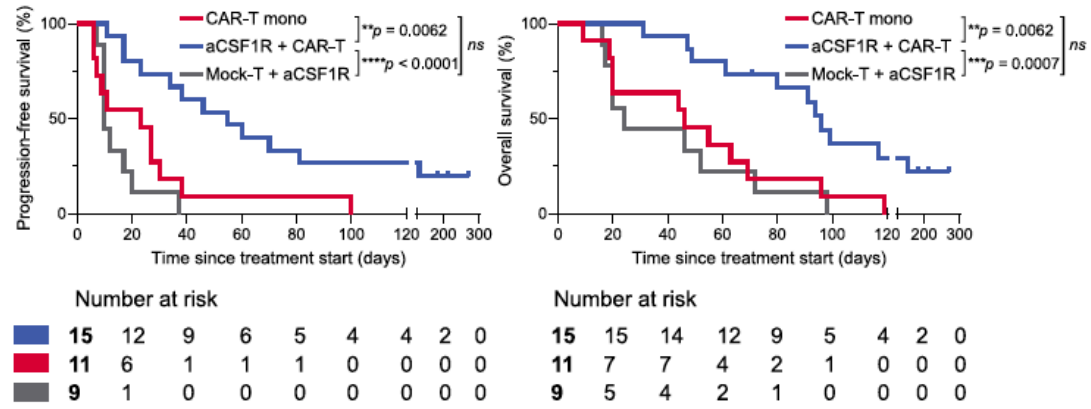






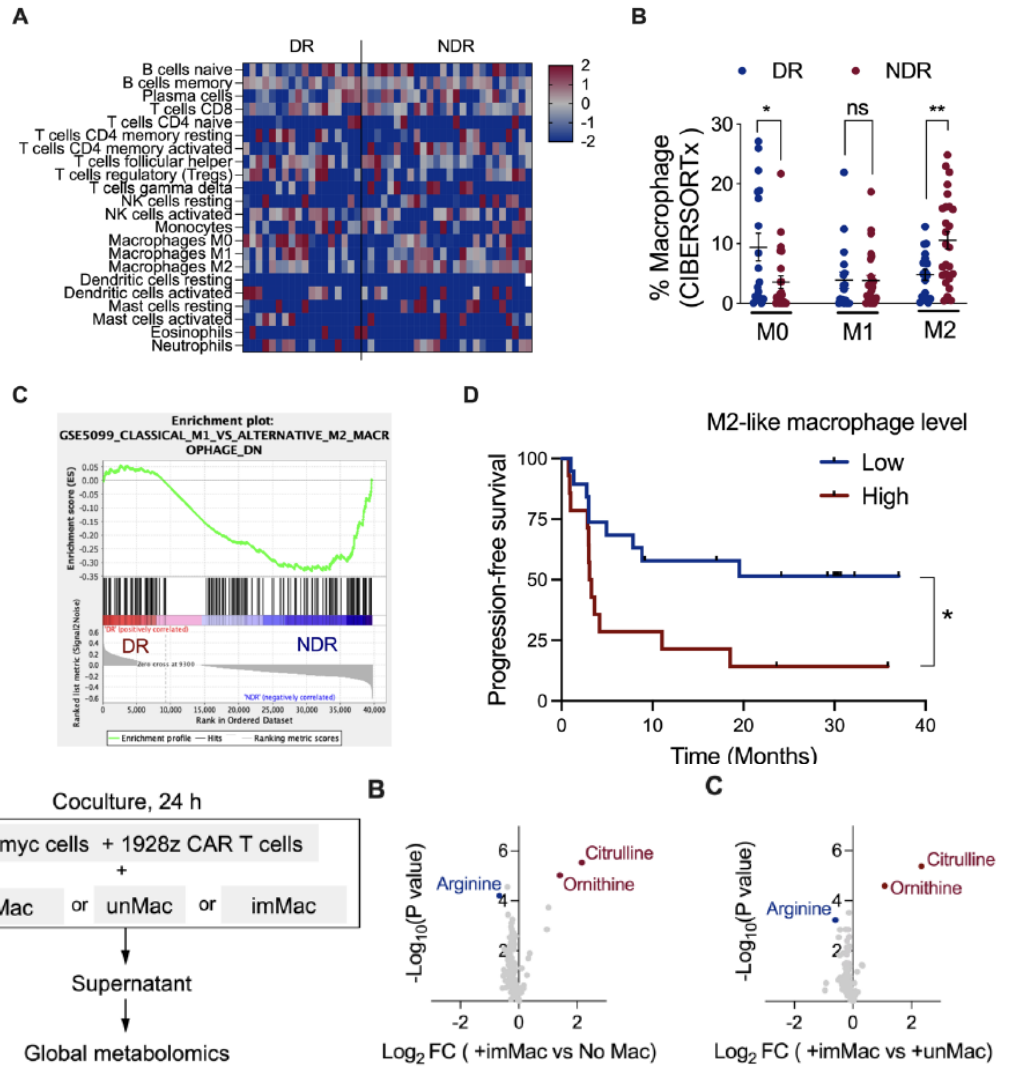






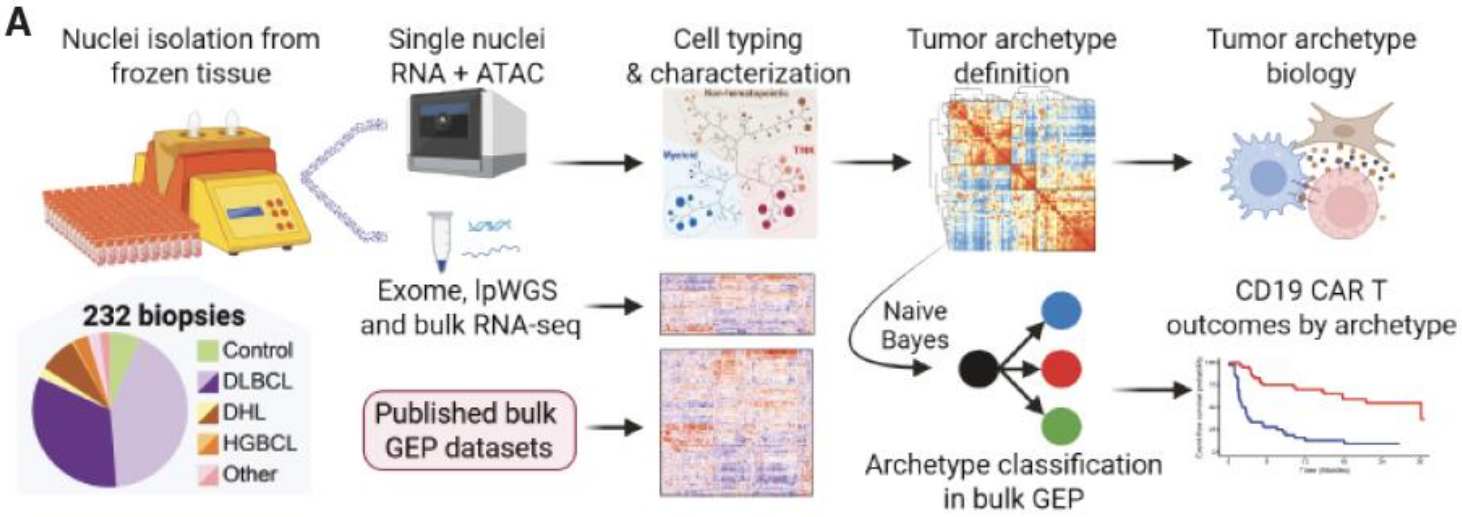
Stahl D et al Cancer Cell 2025;43:1476

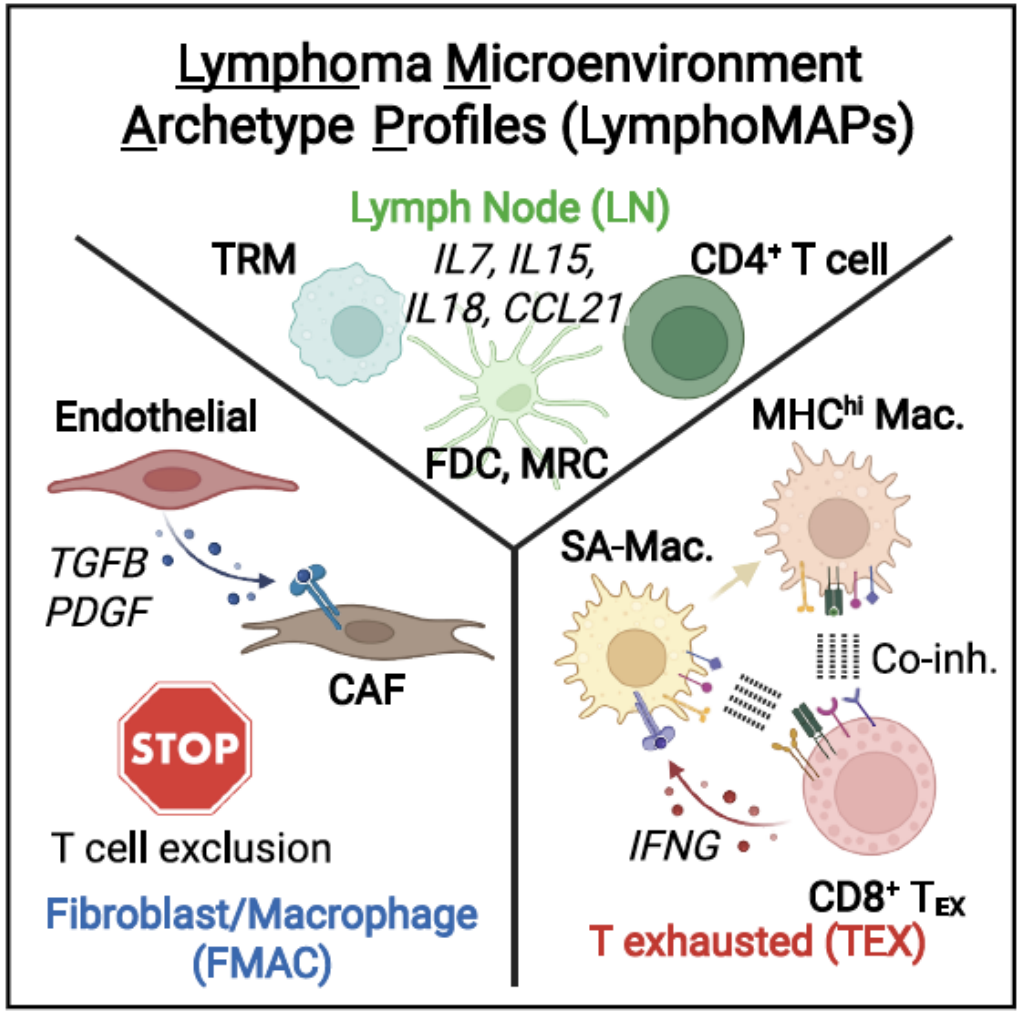
Immunoregulatory Tumoral Macrophages Associate with Poor Outcome after CAR through IFNg-induced iNOS Production



Davila M et al Res Sq [Preprint]. 2025 Mar 31:rs.3.rs-3481746.doi: 10.21203/rs.3.rs-3481746/v1.

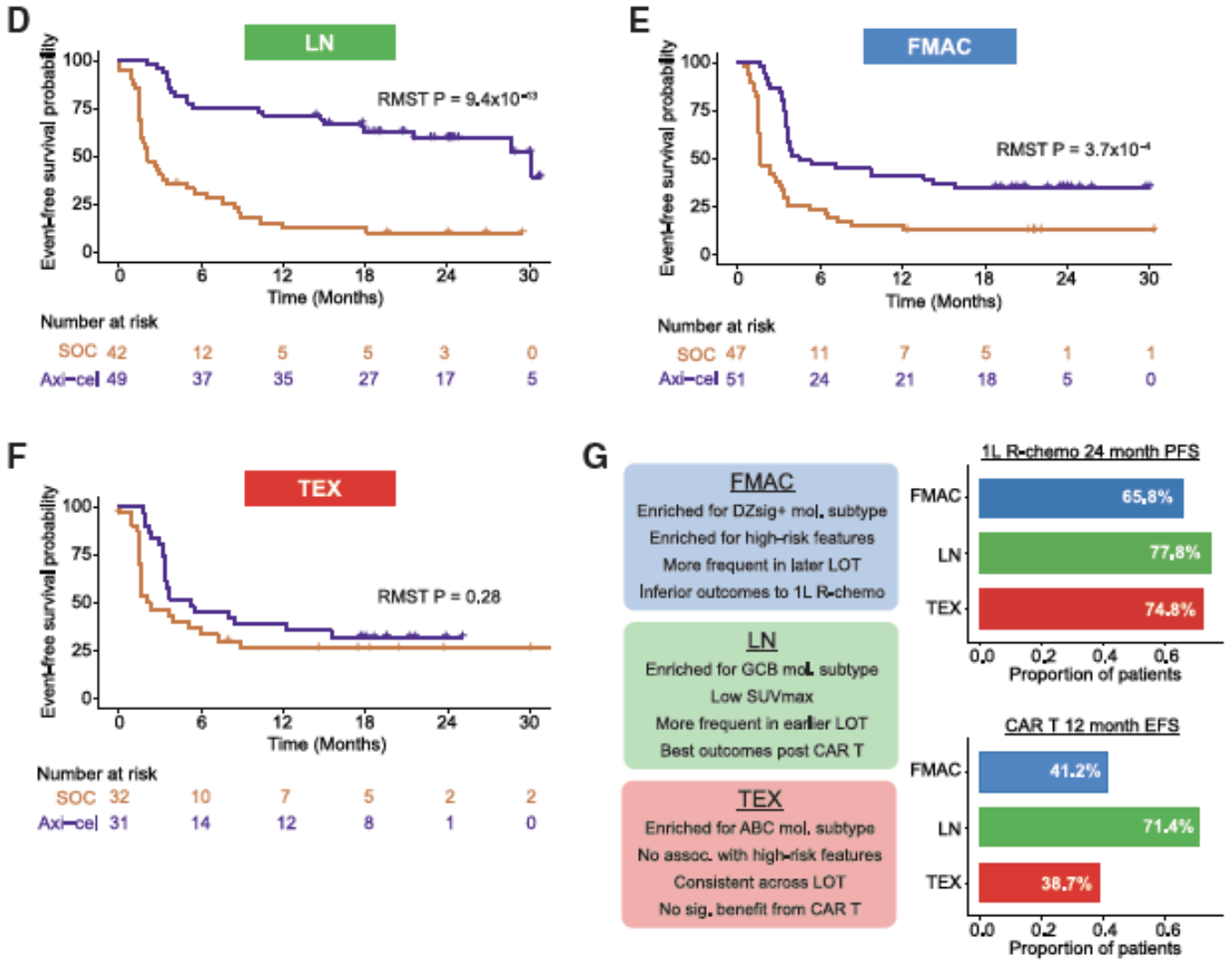
Distinct Lymphoma TMEs Associate with Outcome after CAR





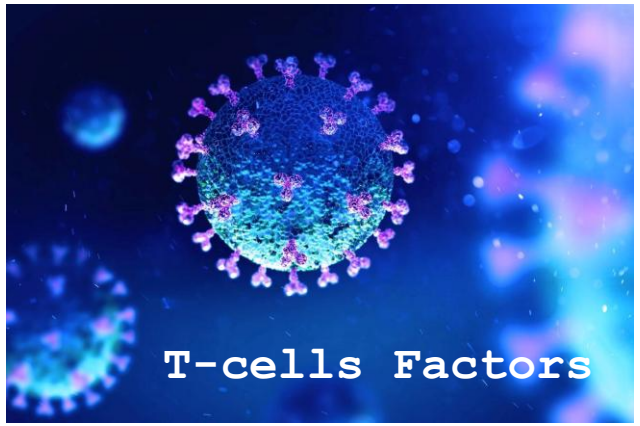
Li X et al Cancer Cell 2025;43:1347

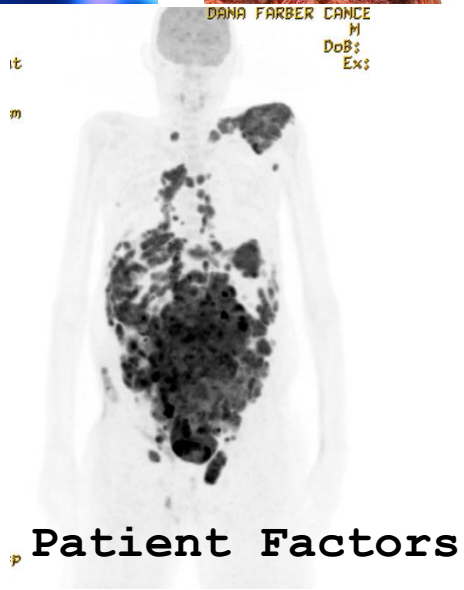
Distinct Lymphoma TMEs Associate with Outcome after CAR



Li X et al Cancer Cell 2025;43:1347

Hypothetical triad of tumor, systemic immune state, and CAR T-cell attributes that influence durable response





Tumor Immune Contexture

CD8+PD-1+LAG-3+/–TIM-3– activated T cell gene signature

Pre-existing Immune System State

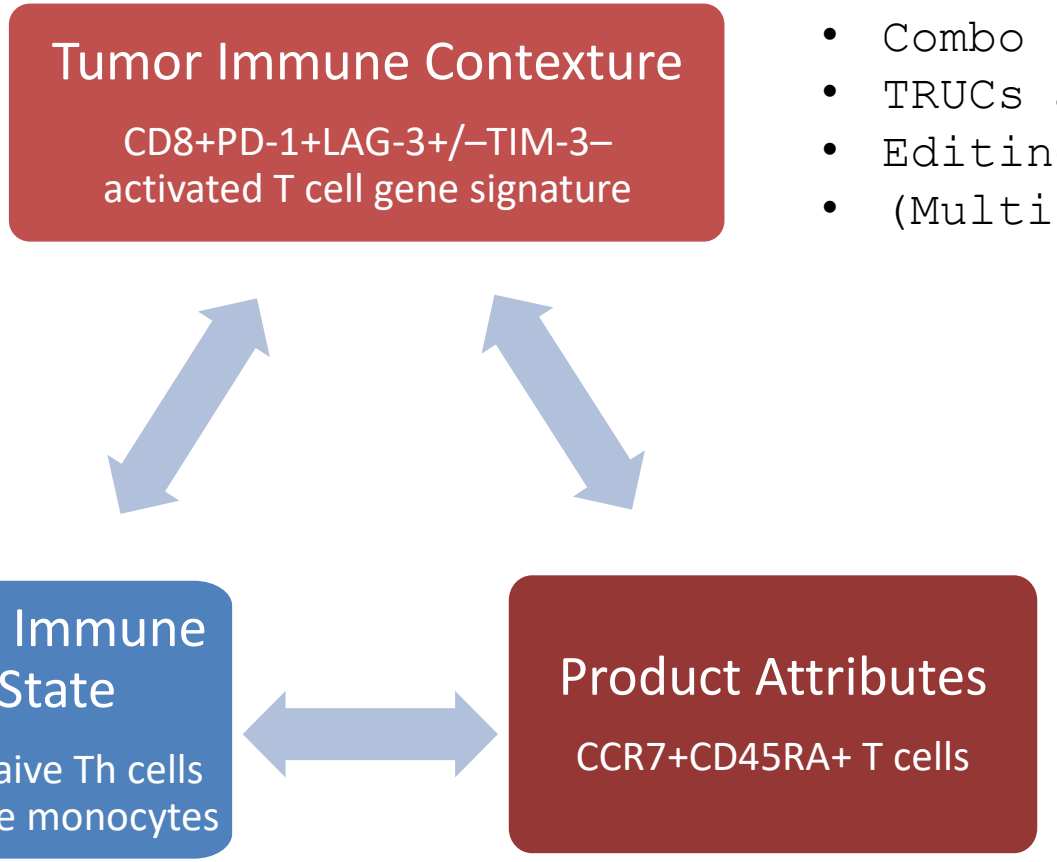
CD27+CD28+ naive Th cells and intermediate monocytes

Product Attributes

CCR7+CD45RA+ T cells

Budka and Chou et al AACR 2021

Previous and Ongoing Attempts to Affect One Aspect of the Triad



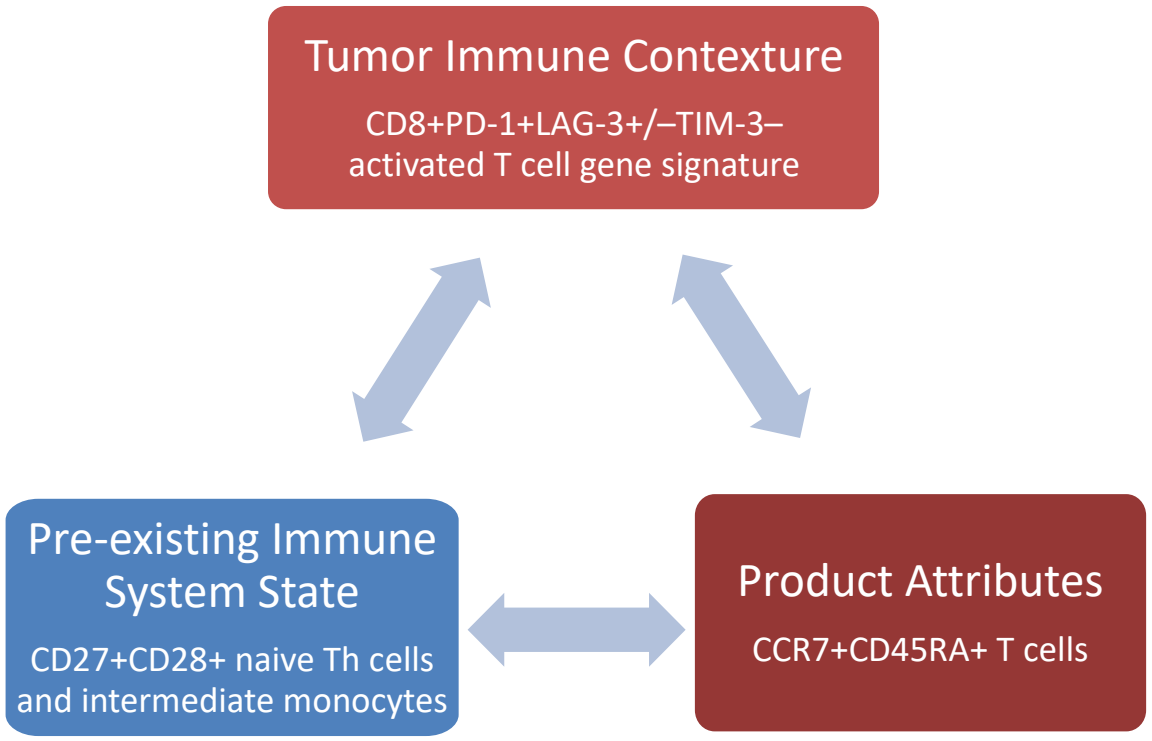
- Combo studies with CPI
- TRUCs and armored CARs
- Editing out inhibitory T-cell receptors
- (Multi-antigen targeting CARs)

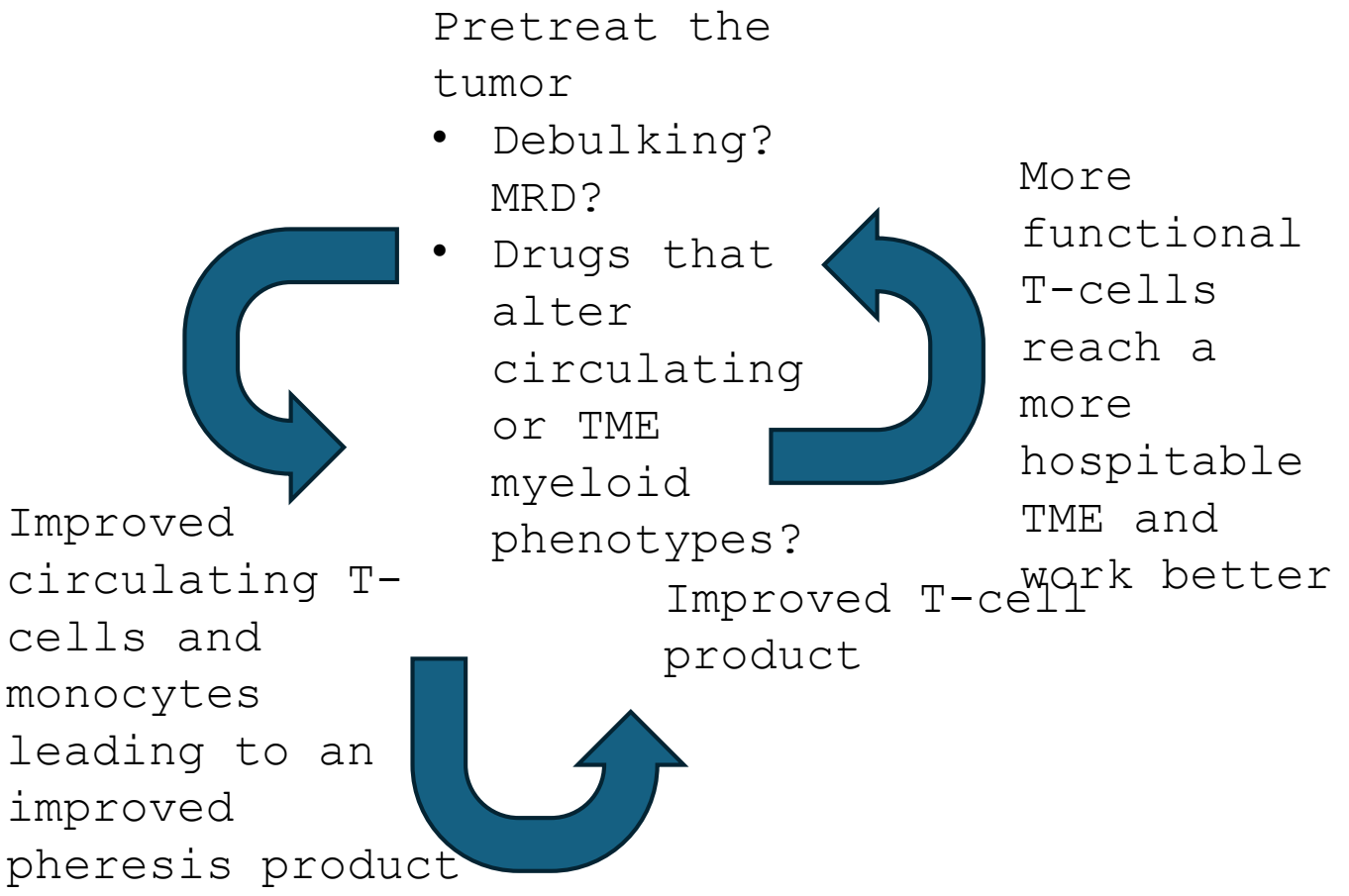
- Pretreatment with immunomodulatory drugs (ie ibrutinib)
- Ex vivo manipulation (ie addition of PI3Ki)
- Shortened manufacturing to improve "stemness"
- Allogeneic CARs
- ? In vivo CARs

- Tumor debulking; CAR for MRD+ CR

Budka and Chou et al AACR 2021

Addressing Determinants of Resistance



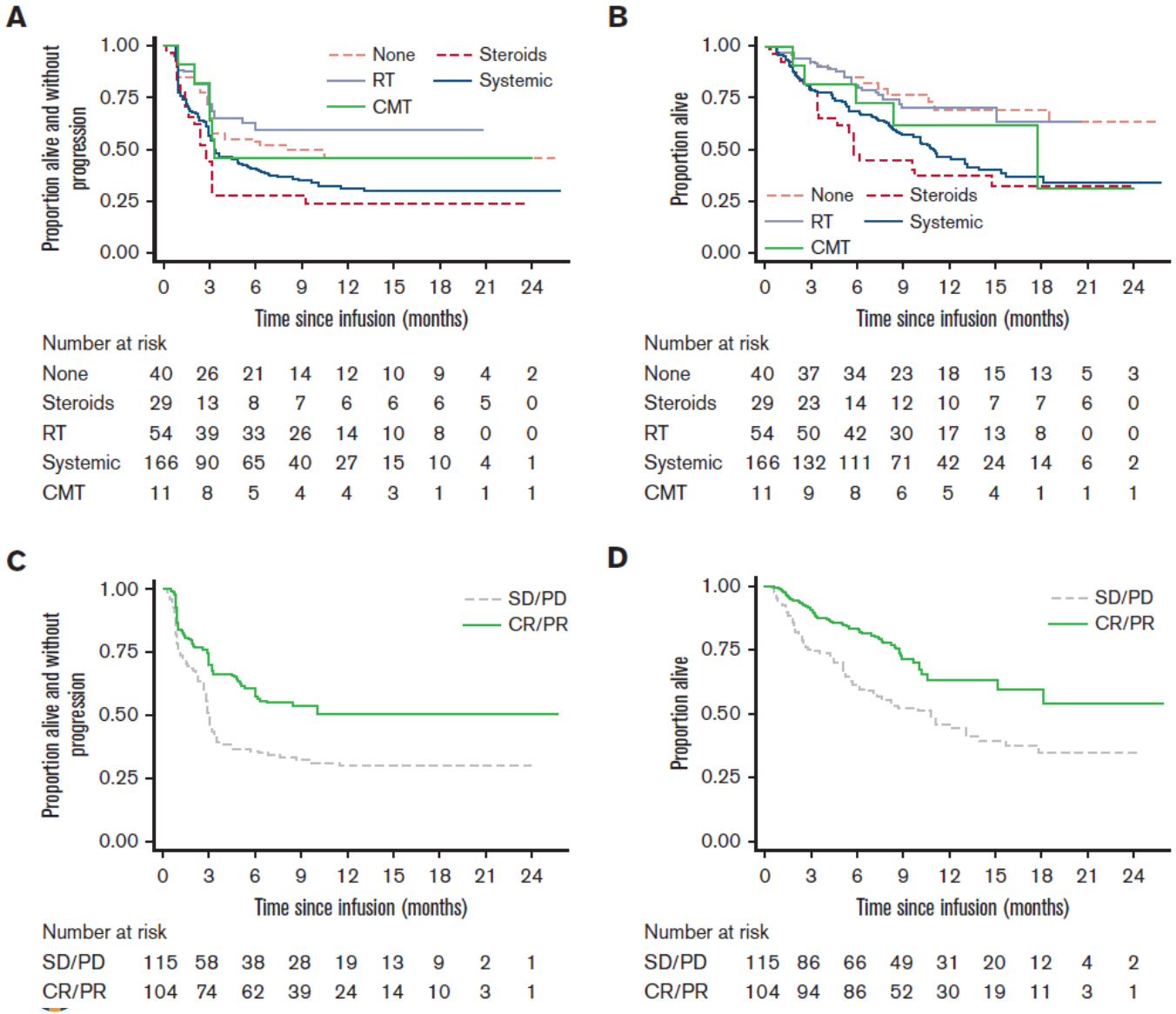


Identify optimal timing of CAR; optimal pretreatment (ideally before pheresis, but also as bridging/conditioning); optimal manufacturing process

Budka and Chou et al AACR 2021

Is Bridging Therapy an Answer, and if so, How Best to Bridge?

Bridging Therapy



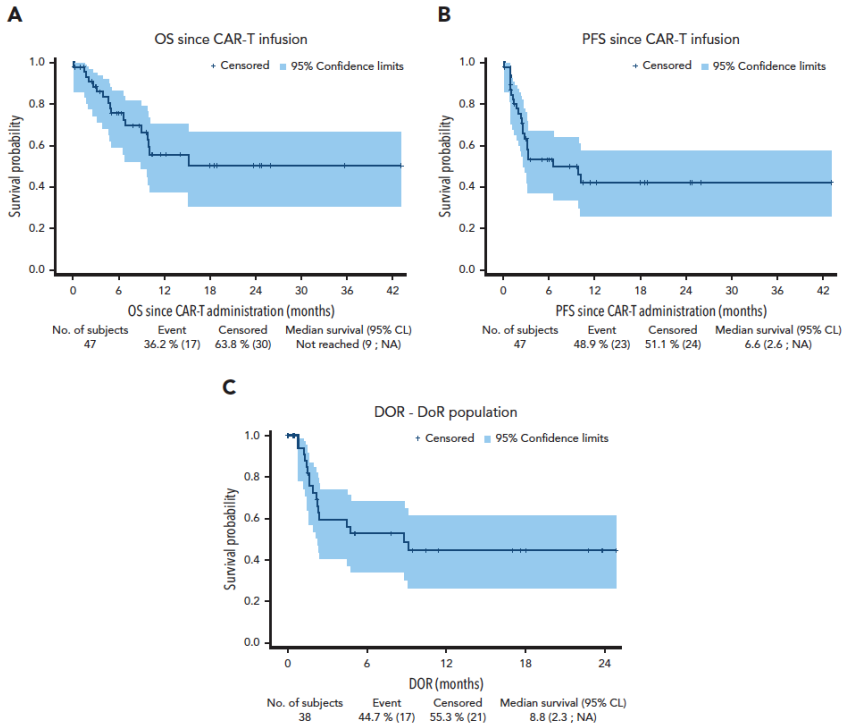
Factors affecting response to BT	Responder/N	OR (95% CI)	*P*-value
RBP bridging			
No	34/134	1.00	.010
Yes	35/83	2.21 (1.21-4.05)	
Response last line			
SD/PD	45/165	1.00	.023
CR/PR	24/52	2.16 (1.11-4.22)	
Bulky disease			
No	55/149	1.00	.045
Yes	14/68	0.49 (0.25-0.98)	

Factors affecting response to CAR-T	Events/N	HR (95% CI)	*P*-value
LDH at LD			
≤2ULN	74/141	1.00	.001
>2ULN	34/41	2.06 (1.34-3.16)	
Extra nodal sites			
<3	91.160	1.00	.001
≥3	17/22	2.51 (1.46-4.32)	
BT response			
SD/PD	68/100	1.00	.012
CR/PR	40/82	0.58 (0.38-0.89)	

Roddie et al Blood Adv 2023; 7:2872

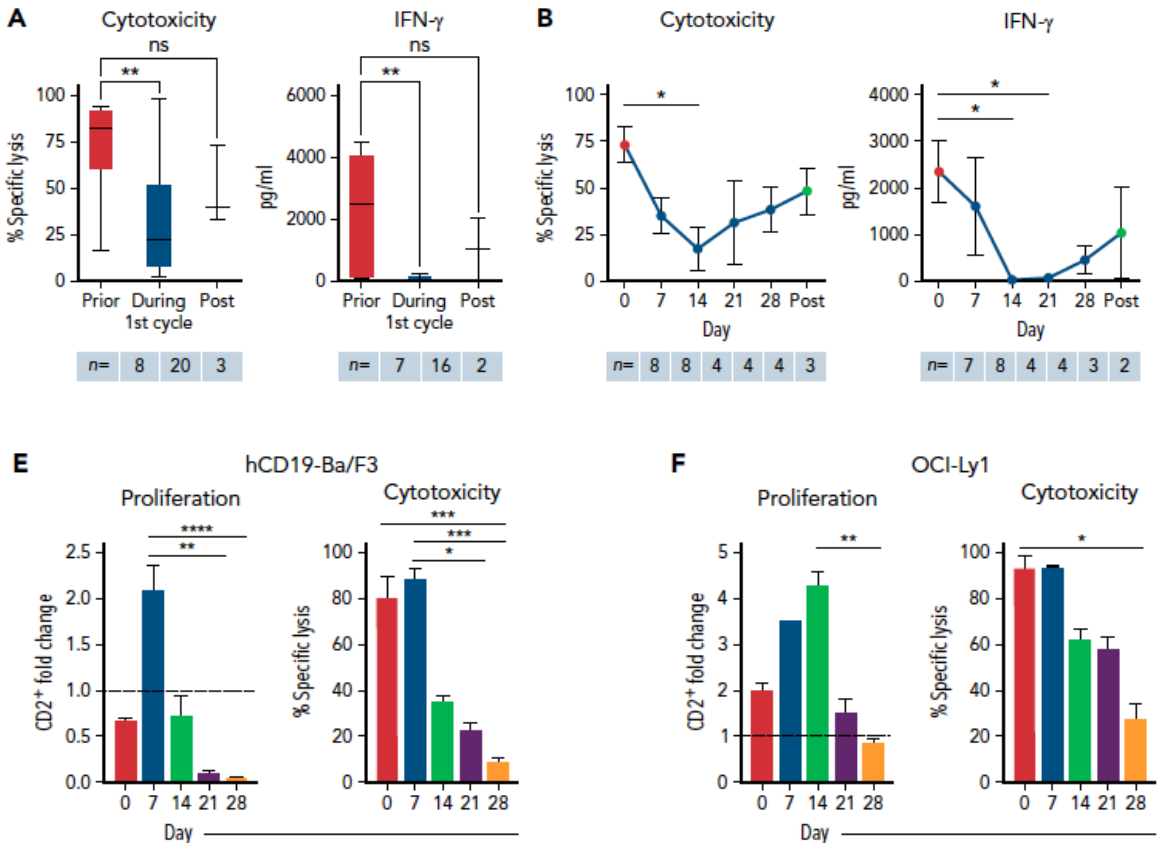
Is Using BiSp Ahead of CAR T-cells OK?

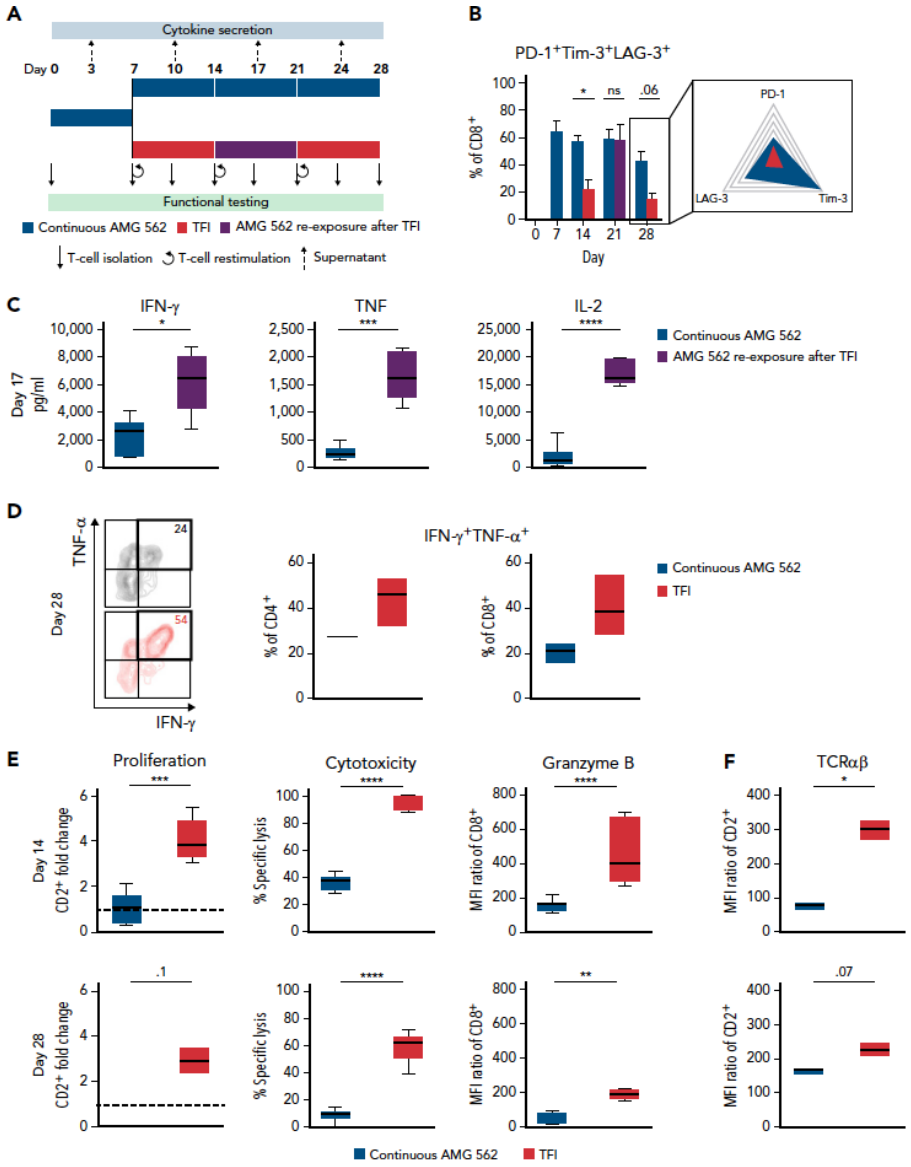
- 47 patients with r/r DLBCL treated with CD19 CAR T-cell therapy who had received prior CD20 (91%) or CD22 (9%) BiSp
- Median time from last dose of BiSp to CAR 51 days; range 13-512 days
  - Outcomes were similar for patients who had received BiSp within 50d compared with those with longer wash-outs
- Conclusion: prior BiSp Ab therapy does not impair CAR T-cell activity but **unclear what defines a sufficient wash-out period**



Crochett Get al Blood 2024;

What is the optimal BiSp washout?

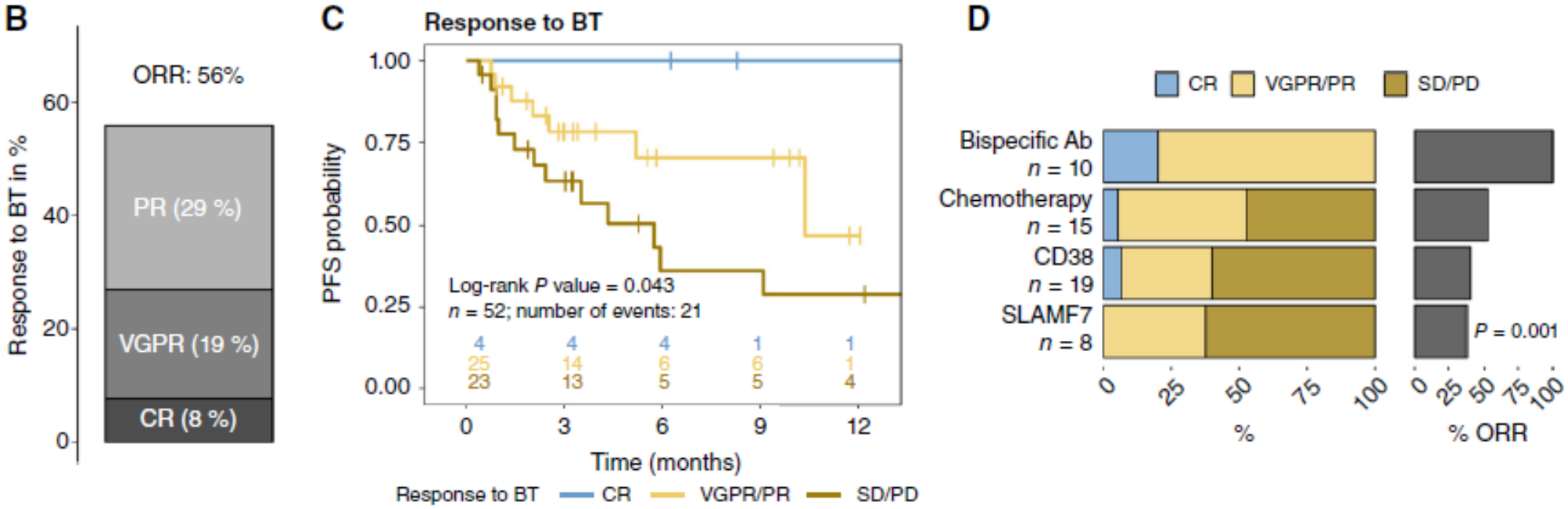




Dana-Farber Cancer Institute

Phillip N et al Blood Adv 2022;14

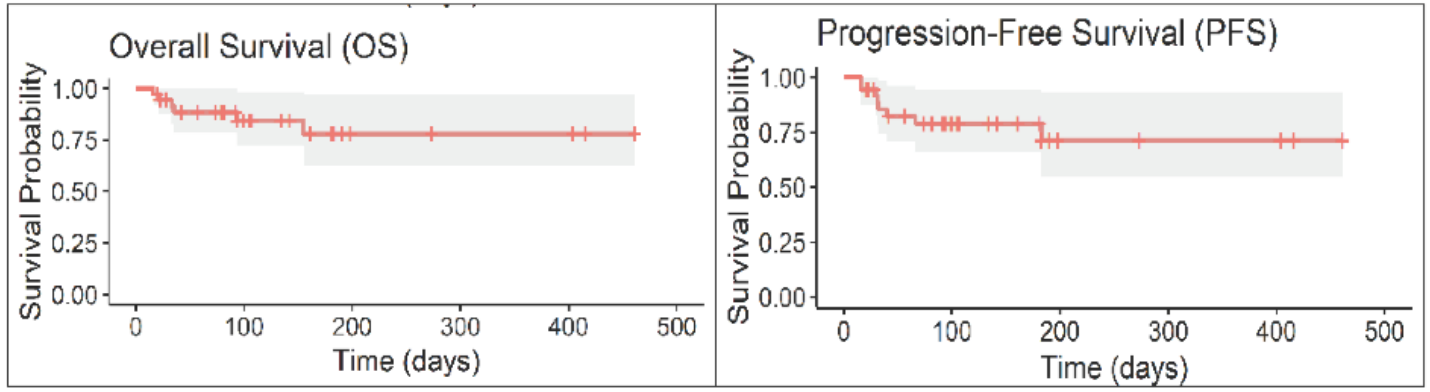
Bridging Therapy: Focus on BiSp Ab



- 10/52 patients got BiSp Ab as bridging therapy pre-BCMA CAR (5 BCMA-CD3, 5 GPRC5D-CD3)
  - **9/10 received BiSp Ab pre-leukapheresis**
  - **BiSp Ab led to the highest rate of OOS products and 3 required recollection**
- BiSp Ab led to the highest ORR compared with other bridging strategies and response to bridging correlated with response to CAR T
- Correlative studies show:
  - Early CD4+ CAR and late CD8+ CAR expansion post BiSp

Fandrei et al Blood Canc Disc 202

Bridging Therapy: Focus on BiSp Ab



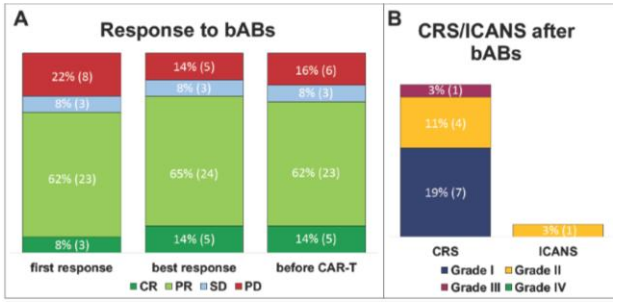
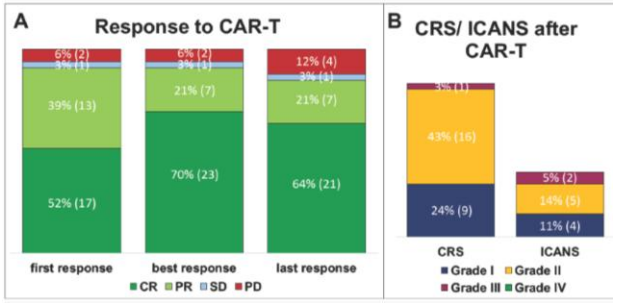


Fig. 1: **A** Response to bABs. **B** Adverse Events (AEs): Data available for 32/37 patients (pts.) 12 pts. with CRS/ICANS. Other reported AEs included neutropenia with sepsis, abdomina pain, and recurrent urinary tract infection.



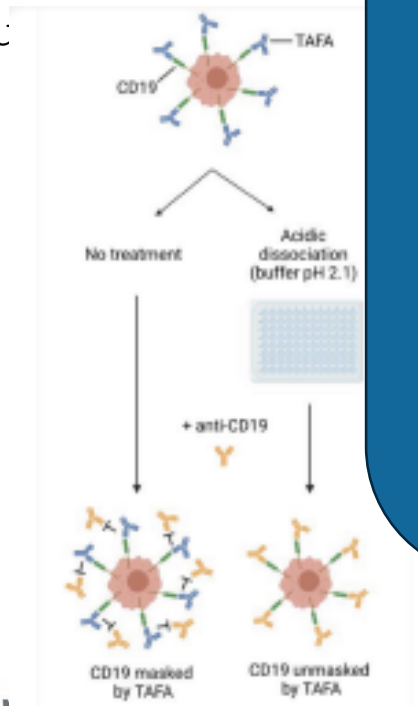
- 37 patients with DLBCL bridged with bispecifics to CAR (axi-cel, liso-cel, rare tisa-cel)
  - 7 received BiSp Ab pre-leukapheresis
  - 33 received BisP Ab as bridging
- Data was not separated by holding vs bridging but when considering outcomes for the group as a whole:
  - ORR 56%, CR 14% to BiSp as bridging
  - ORR 83%, CR 70% to CAR
  - 1 year PFS ~75% post-CAR
- CRS and ICANS rates were comparable to historical controls

Zeiger et al ICML 2025

Is Using A CD19 Targeted Therapy Ahead of CAR T-cells OK?

Tafasitamab:

- Concern for epitope masking requiring a wash-out period more than antigen loss
- One series of 9 patie
  with tafa/R^2 Immediat
  of CD19 CAR (median
  4m) resu
  PRs.



Loncastuximab:

- Less concern for epitope masking and/or antigen loss
- Small cohort of patients s/p
  T or bridging
  ORR and
  se durability

onca Therapy Type		
t T 5	Bridging Therapy n = 11	Total N = 16
	64	63
	36	44

: 33%
: 28%

ridging therapy: 43 days
a as their last LOT: 57 days
Median duration from end of Lonca treatment to CAR-T: 16 days

What about CD19 BiSp ahead of CAR?

May breed a phenotype of relapsed DLBCL that cannot be salvaged…

But is the objective to cure the most in 1L or maintain salvagability in 2L+?

Illieva K et al Front Immunol 2023;14:1274556; Epperla N et al Biomark Res 2025;13:18; Hamadani M et al eJHaem 202

Polatuzumab in 2L: Polatuzumab-R-GemOx (POLARGO)

Key eligibility criteria

- DLBCL, NOS or history of transformation of indolent disease to DLBCL
- R/R disease after ≥1 prior line of treatment
- Ineligible for transplant

Safety run-in
*Enrolled n=**15***

→ ***Pola-R-GemOx**** *Q3W up to 8 cycles*

Primary endpoint
Safety and tolerability

Randomized phase
*Enrolled n=**255***

→ R 1:1

→ ***Pola-R-GemOx**** *n=129 Q3W up to 8 cycles*

→ ***R-GemOx*** *n=126 Q3W up to 8 cycles*

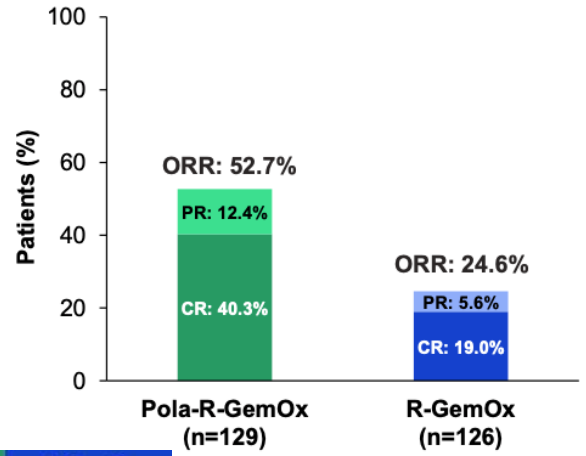
Primary endpoint
OS

Key secondary endpoints
PFS (by INV)
CR† (by IRC)
ORR† (by IRC)

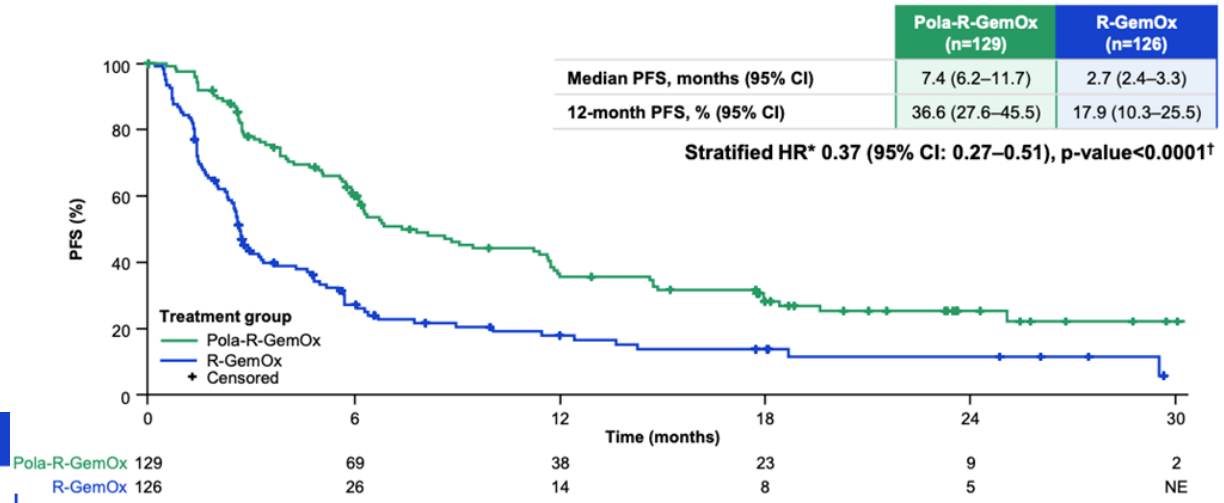
Stratification Factors
- Age (≤70 vs >70 years)
- Prior lines of therapy (1 vs ≥2)
- Relapsed vs refractory

n (%)	Pola-R-GemOx (n=128)	R-GemOx (n=125)
Thrombocytopenia*	68 (53.1)	51 (40.8)
Grade ≥3	44 (34.4)	33 (26.4)
Neutropenia*	53 (41.4)	52 (41.6)
Grade ≥3	43 (33.6)	38 (30.4)
Febrile neutropenia†	3 (2.3)	3 (2.4)
Grade ≥3	3 (2.3)	3 (2.4)
Anemia*	48 (37.5)	35 (28.0)
Grade ≥3	17 (13.3)	19 (15.2)
Infections*	53 (41.4)	39 (31.2)
Grade ≥3	28 (21.9)	12 (9.6)
Hepatic toxicity*	41 (32.0)	25 (20.0)
Grade ≥ 3	11 (8.6)	2 (1.6)

Response rates by PET-CT at EOT (IRC-assessed)

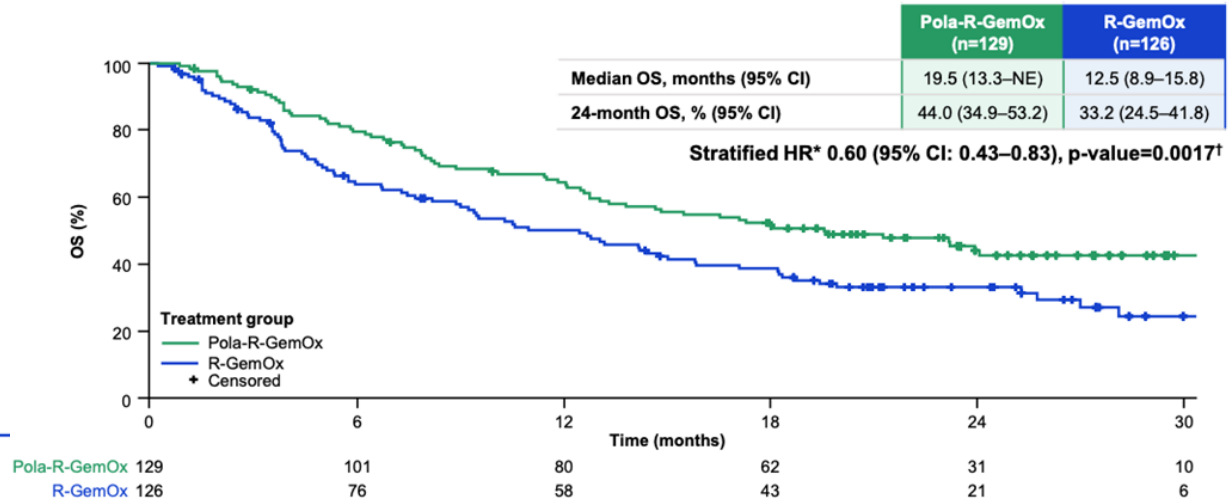


n (%), unless otherwise specified	Pola-R-GemOx (n=128)	R-GemOx (n=125)
Any Grade PN*	73 (57.0)	36 (28.8)
Grade 1	48 (37.5)	29 (23.2)
Grade 2	20 (15.6)	7 (5.6)
Grade 3	5 (3.9)	0



	Pola-R-GemOx (n=129)	R-GemOx (n=126)
Median PFS, months (95% CI)	7.4 (6.2–11.7)	2.7 (2.4–3.3)
12-month PFS, % (95% CI)	36.6 (27.6–45.5)	17.9 (10.3–25.5)

Stratified HR* 0.37 (95% CI: 0.27–0.51), p-value<0.0001†



	Pola-R-GemOx (n=129)	R-GemOx (n=126)
Median OS, months (95% CI)	19.5 (13.3–NE)	12.5 (8.9–15.8)
24-month OS, % (95% CI)	44.0 (34.9–53.2)	33.2 (24.5–41.8)

Stratified HR* 0.60 (95% CI: 0.43–0.83), p-value=0.0017†

Matasar et al EHA 2025

Bridging Therapy: Recommendations

- Avoid bridging therapy as able
- If bridging therapy is needed, choose a therapy that has the best chance of leading to a response while minimizing post-CAR toxicity
  - RT is probably best; favor pola-Rgemox if pola-naïve or previously responsive, and systemic therapy is needed
  - BiSp, especially AFTER pheresis, are probably safe and effective
  - Still unclear is how to use drugs that target the same target as the CAR T-cell
- Bridging therapy may be most important for products with longer manufacturing timelines and/or less potent CAR T-cell expansion

Holding Therapy: Recommendations

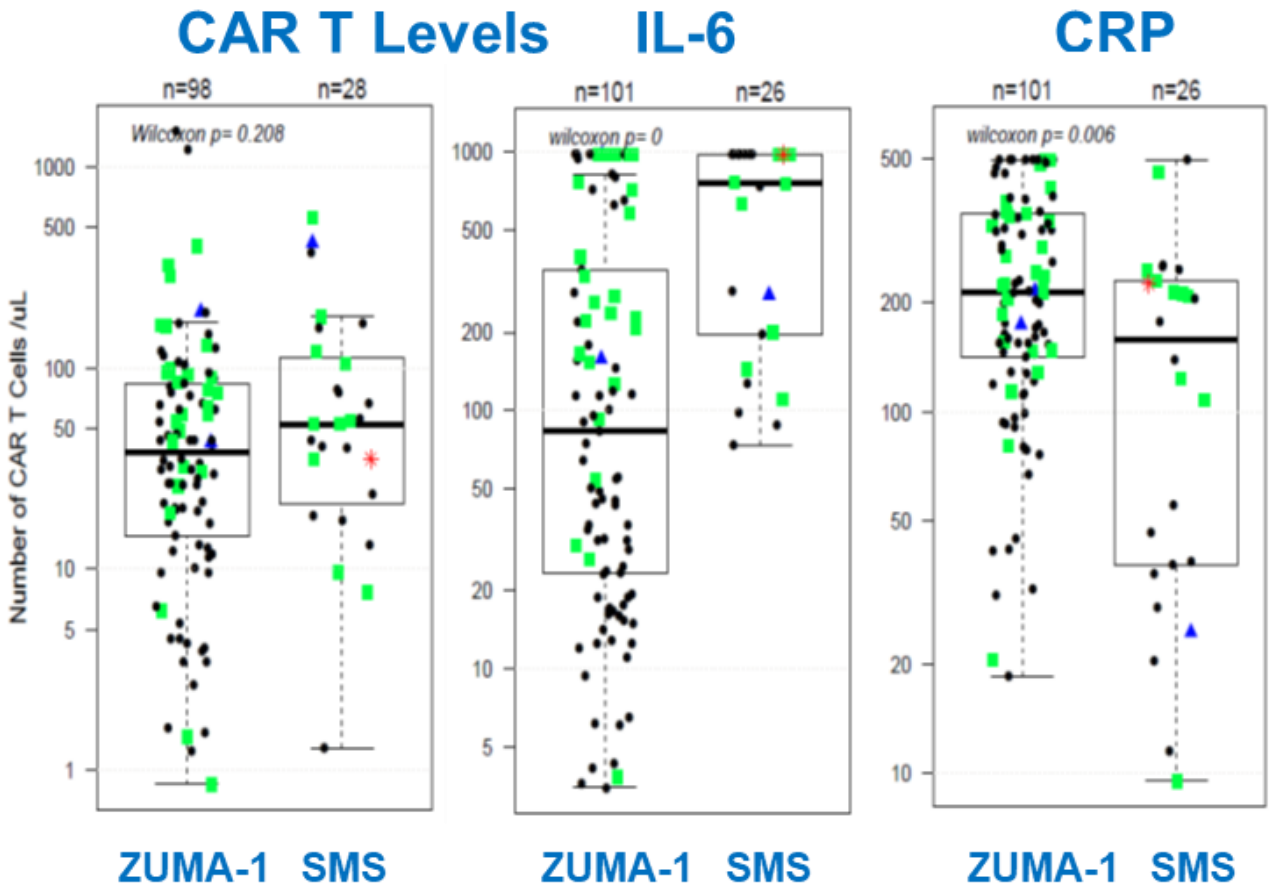
- Encourage community referrers to refer all patients at least one line of therapy before CAR is needed; high-risk patients even earlier
  - For CAR in the second line, refer all high-risk patients at or around diagnosis (also helpful for considering frontline trials)

- If holding therapy is needed, choose a therapy that has the best chance of leading to a response while minimizing T-cell toxicity
  - RT is probably best
  - BiSp pre-leukapheresis could negatively influence T-cell health/function

Once You Have Selected and Optimized Your Patient, Can Care be Further Optimized?

Prophylactic Toci Improves CRS but Worsens ICANS

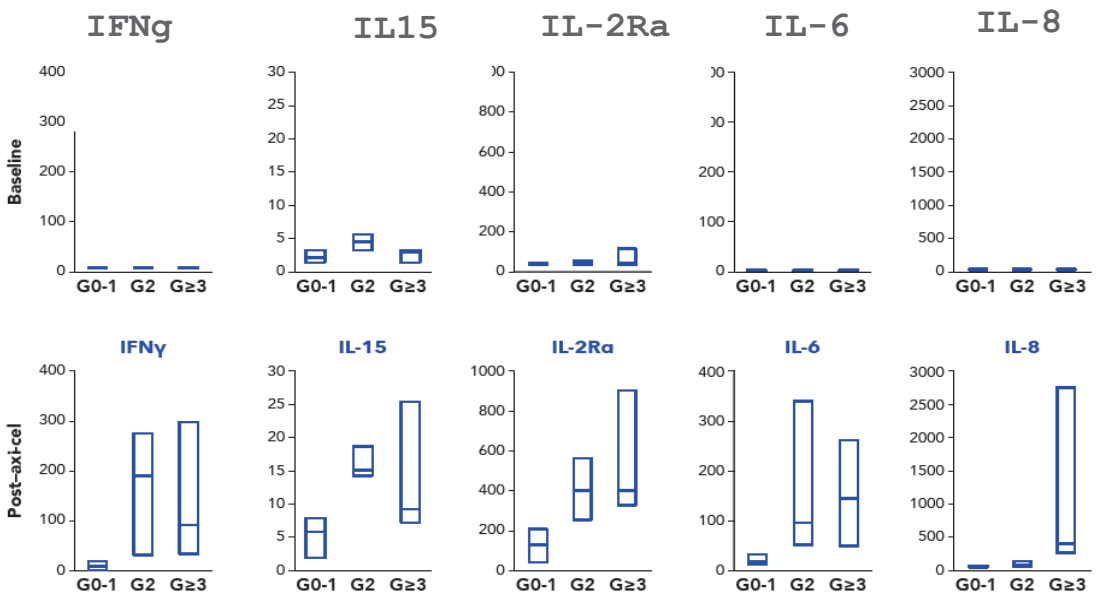
- All patients received a dose of tocilizumab on day 2 (N=34)
- Median peak CAR: 52.6 (vs 41.9 Z1)
- Median AUC CAR: 570.5 (vs 462.3 Z1)
- Grade 3+ CRS: 3% (vs 13% Z1)
- Grade 3+ NT: 41% (vs 28% Z1)



Locke et al ASH 2017; Locke et al Transplant Cell Thera

Prophylactic Toci may worsen ICANS via increased CSF myeloid cells and cytokines

Pre- and post-axicel CSF cy

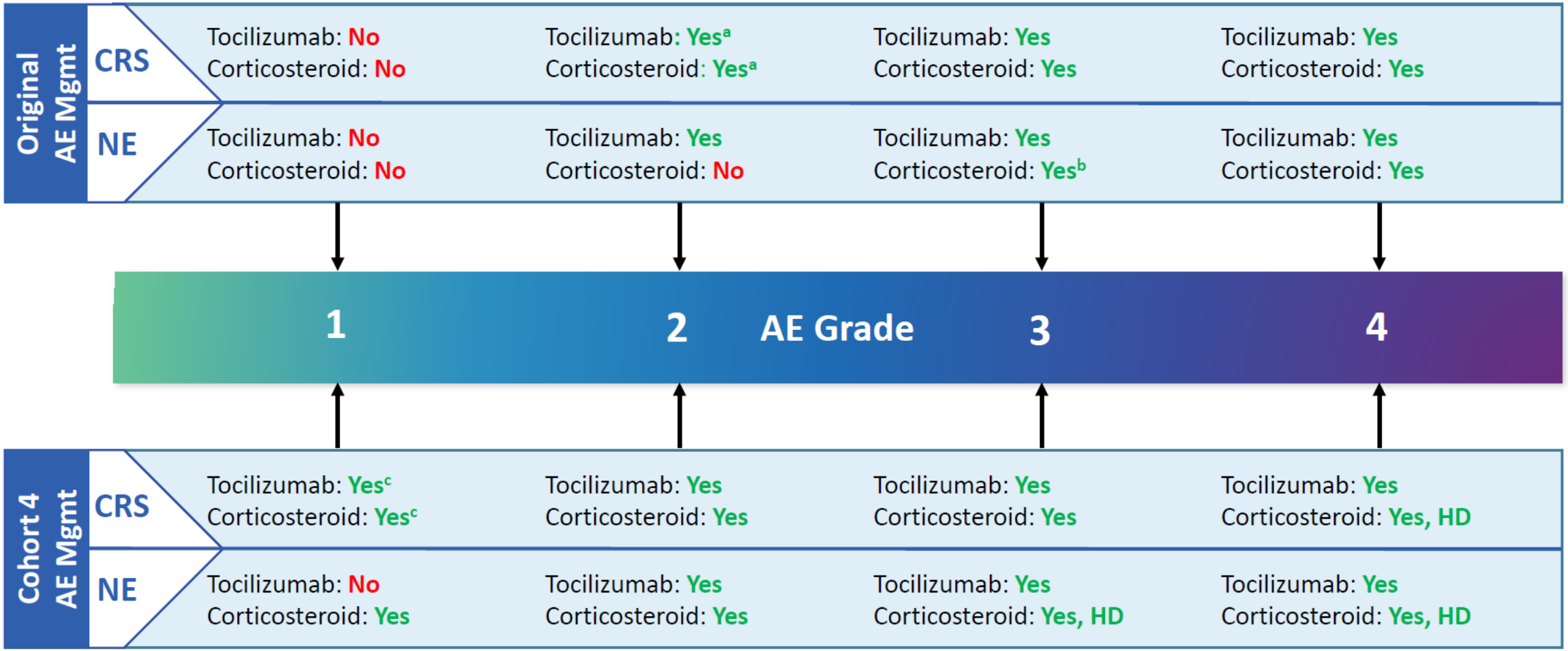


Post-axicel CSF mononuclear cells

Cell Populations	NE Grade 0-2 (N = 15) Median cells/mL (Q1, Q3)	NE Grade ≥ 3 (N = 10) Median cells/mL (Q1, Q3)	NE Grade ≥ 3/ Grade 0-2 Ratio
~~CD45+ (leukocytes)~~	~~166 (18, 1504)~~	~~570 (170, 1700)~~	~~3.4~~
CD14+ (myeloid cells)	18 (11, 436)	306 (125, 1063)	17
~~CAR+ T cells~~			
CD3+	35 (6, 324)	79 (47, 81)	2.3
CD4+	26 (3, 213)	52 (34, 65)	2
CD8+	17 (1, 85)	15 (10, 34)	0.9
CD4/CD8 T cell ratio	2 (1, 5)	3 (2, 5)	1.6
CAR+ CD4/CAR CD8 T cell ratio	9 (8, 53)	17 (11, 22)	2

Locke et al ASH 2017; Locke et al Transplant Cell Thera

Early and Prophylactic Steroids Improves CRS and ICANS without Sacrificing Efficacy

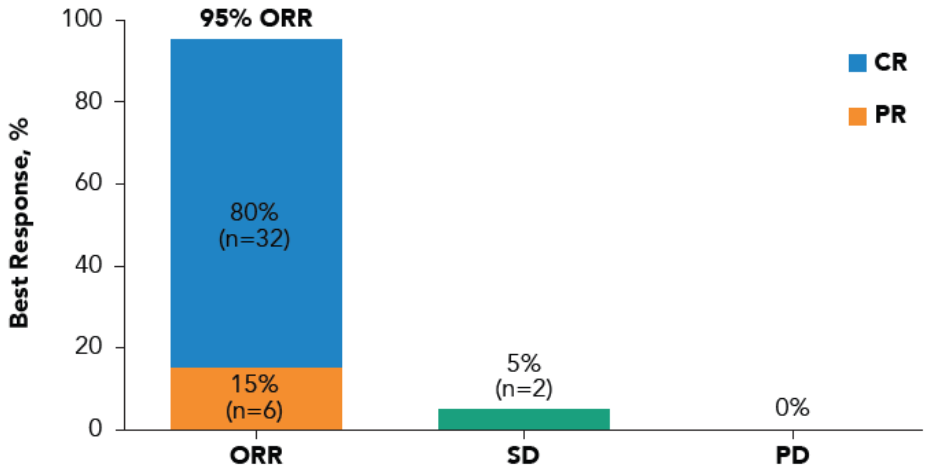


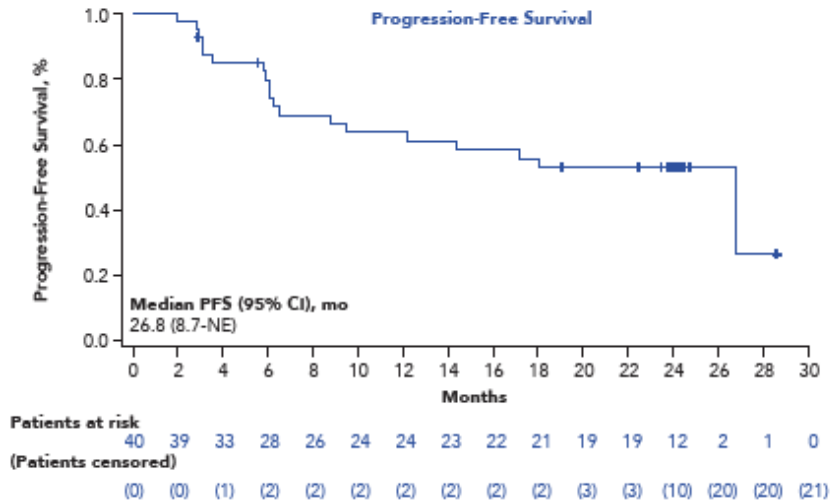
Oluwole et al ASH 2021

Early and Prophylactic Steroids Improves CRS and ICANS without Sacrificing Efficacy

	Cohort 6 (N=40)
CRS, n (%)	**32 (80)**
Worst Grade 1	14 (35)
Worst Grade 2	18 (45)
Worst Grade ≥3	0 (0)
Median time to onset (range), days	5 (1–15)
Median duration (range), days	4 (1–11)
Neurologic event, n (%)	**23 (58)**
Worst Grade 1	10 (25)
Worst Grade 2	7 (18)
Worst Grade ≥3	6 (15)
Median time to onset (range), days	6 (2–162)
Median duration (range), days	19 (1–438)

Median (Q1, Q3)	Cohorts 1+2 Overall (N=101)	Cohort 6 Overall (N=40)	Cohorts 1+2 After Matching (n=32)	Cohort 6 After Matching (n=32[a])
Peak CAR T-cell levels				
CAR T-cell expansion, cells/μL	38 (15, 83)	64 (6, 131)	43 (14, 107)	65 (18, 146)
Peak cytokine levels				
IFN-γ, pg/mL	477 (196, 1097)	208 (87, 446)	481 (120, 1096)	227 (103, 424)
IL-2, pg/mL	22 (10, 38)	8 (3, 23)	23 (10, 58)	8 (3, 16)
GM-CSF, pg/mL	7 (2, 16)	2 (2, 5)	9 (2, 21)	2 (2, 4)
Ferritin, ng/mL	3001 (1326, 6683)	904 (489, 1529)	2312 (1225, 4777)	809 (489, 1529)
CRP, mg/L	214 (141, 353)	76 (39, 136)	175 (124, 345)	78 (44, 131)



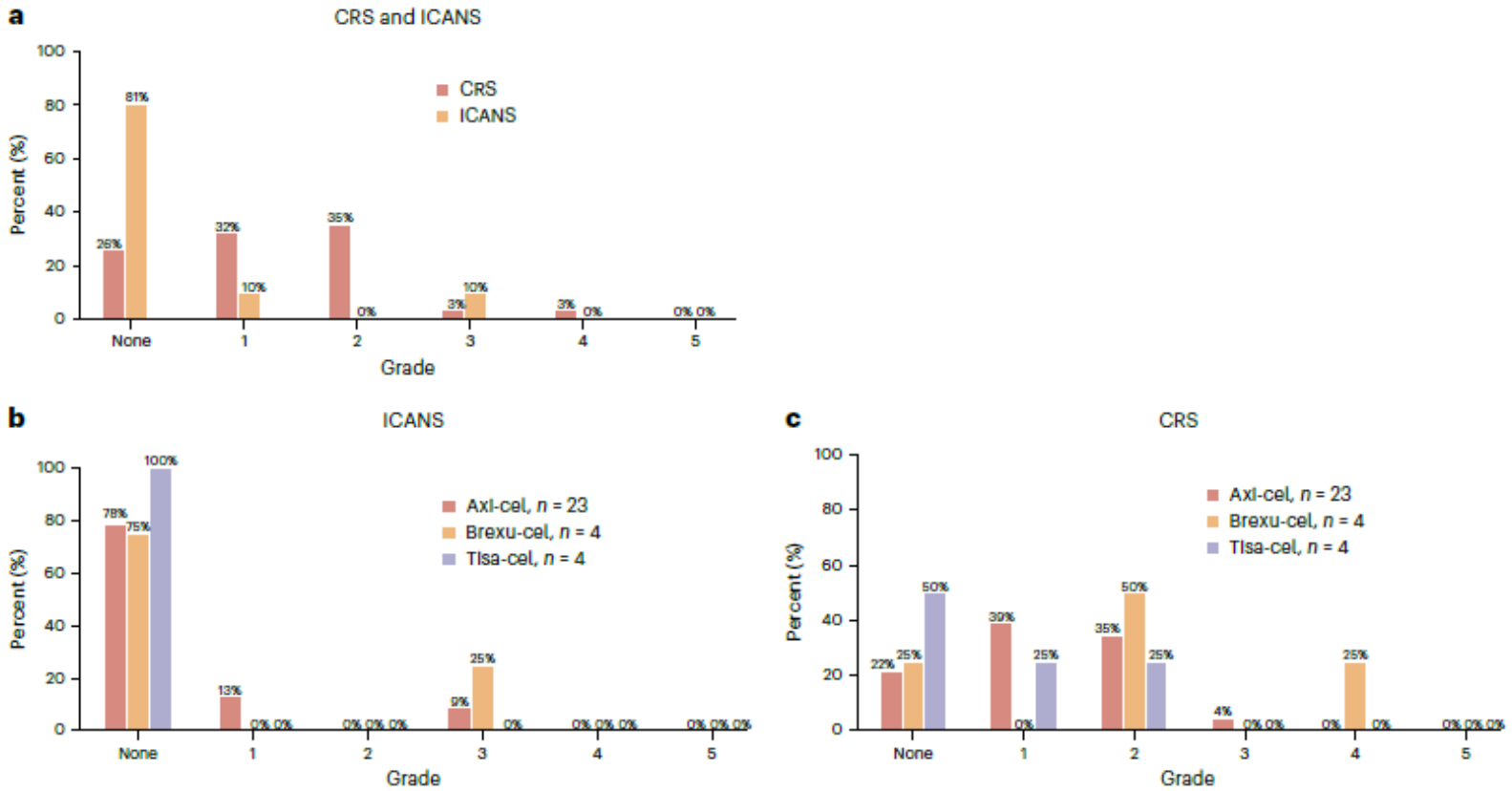


Oluwole et al ASH 2021, 2022; Bone Marrow Transplant 2024

※使用にあたっては日本の添付文書に従うこ

Prophylactic Anakinra produces discrepant results across clinical trials: MSKCC study WITH clinical benefit

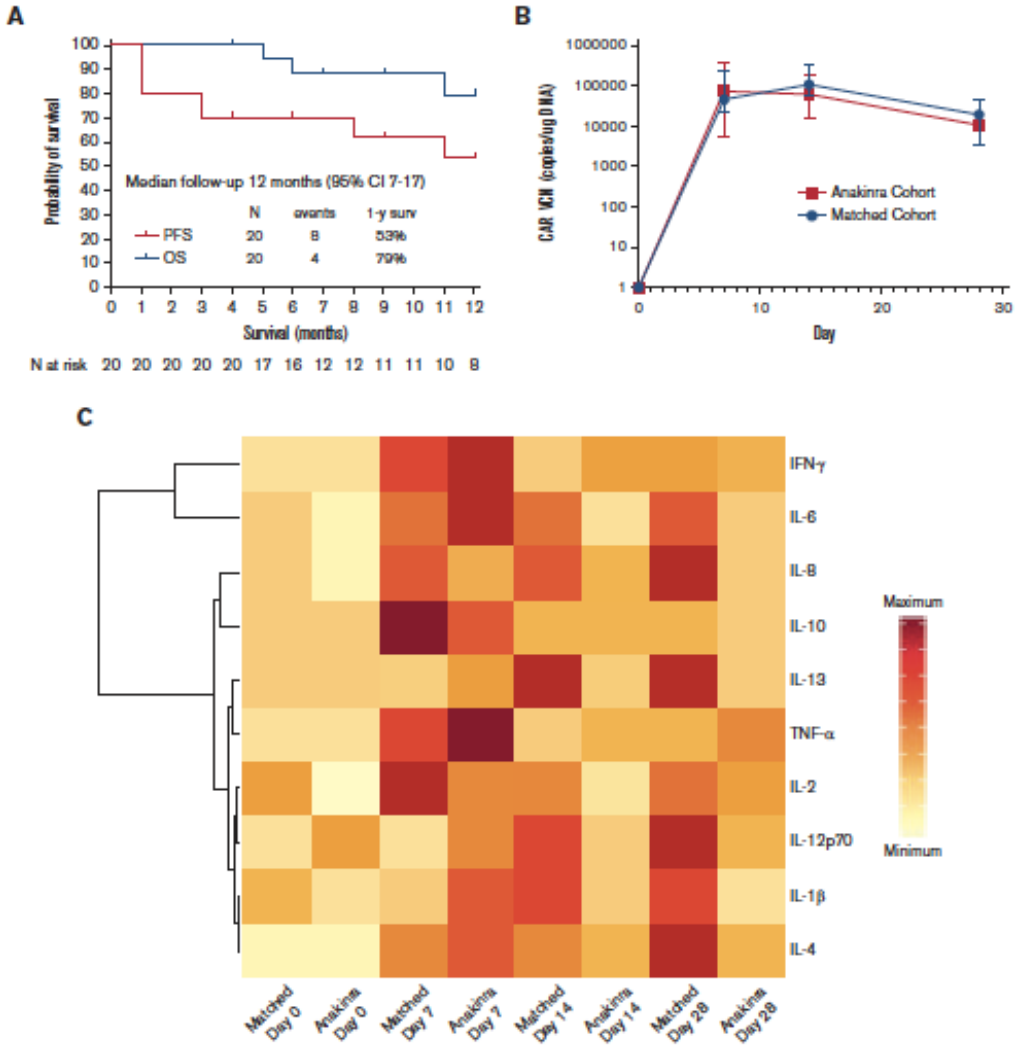
- N=31 (axi-cel 23; brexu-cel 4; tisa-cel 1)
  - All with LBCL or MCL
- Patients received SC anakinra D2-10; 100mg bid-qid
- No alteration in clinical efficacy
- CRS
  - Any grade: 74%
  - High-grade: 6%
- ICANS



Park et al Nature Med 2023;29:1710

※イエスカルタの添付文書の効能・効果を参

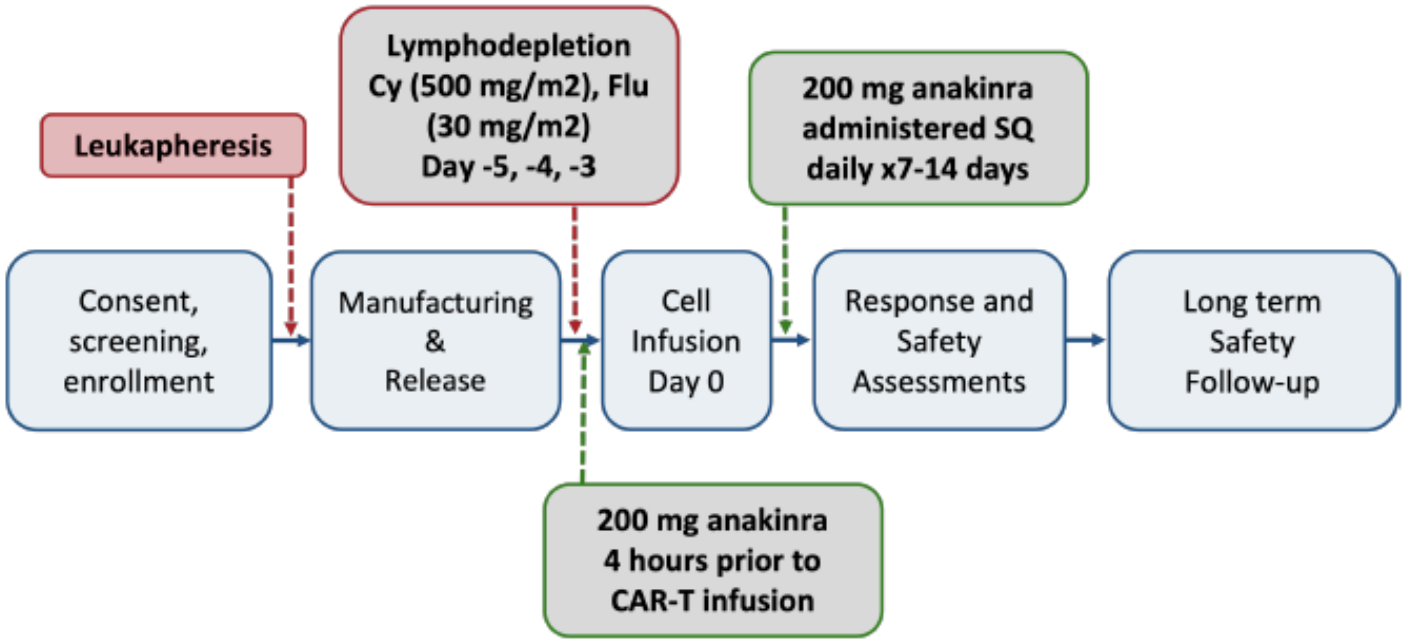
Prophylactic Anakinra produces discrepant results across clinical trials: MD Andersen study WITH LIMITED clinical benefit



- N=20 patients, all received axi-cel
- CAR T-cell expansion and efficacy was not impacted by ppx anakinra
- Rates of CRS and ICANS were not reduced
  - CRS:
    - Any grade CRS: 95%
    - High-grade CRS: 5%

Strati P et al Blood Adv

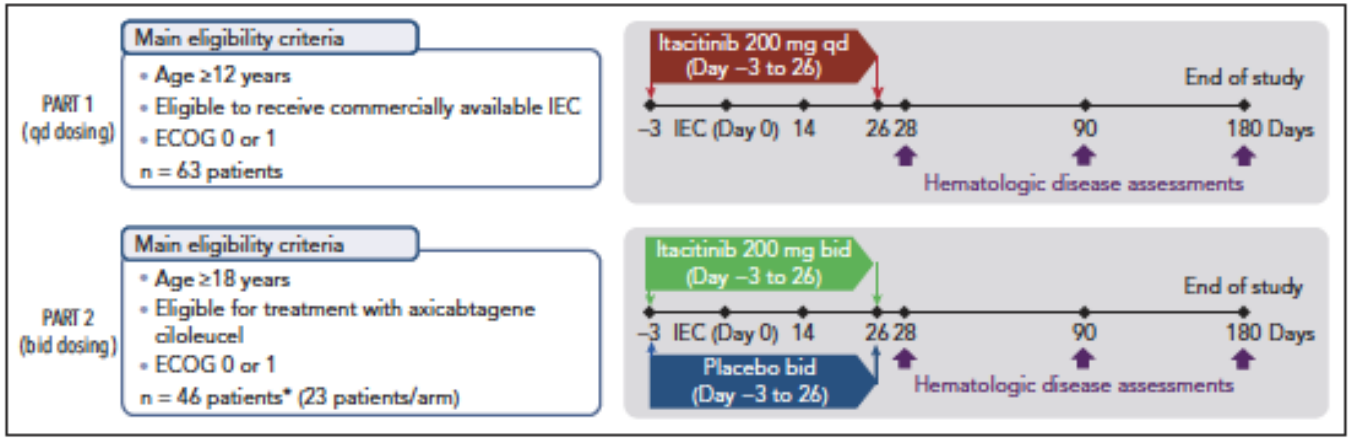
Prophylactic Anakinra produces discrepant results across clinical trials: MGH study WITHOUT clinical benefit

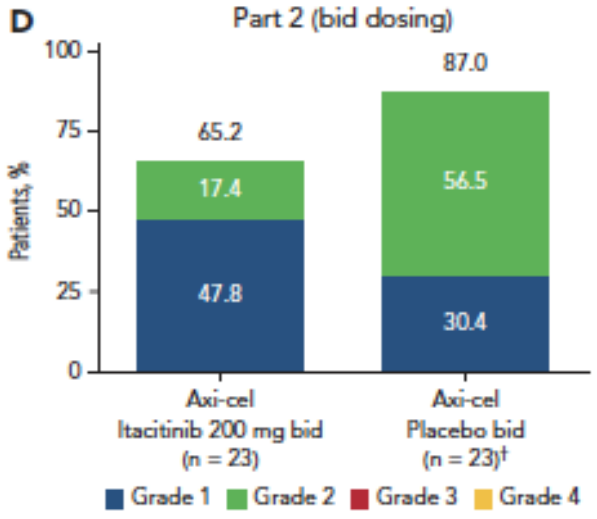


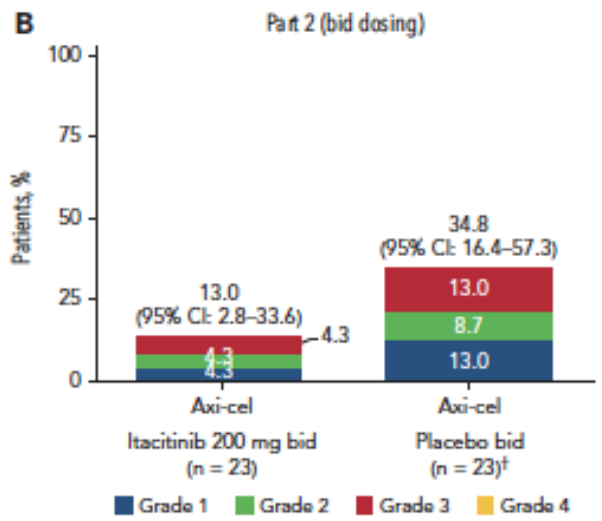
- N=15 patients, all received axi-cel
- CAR T-cell expansion and efficacy was not impacted by ppx anakinra
- Rates of CRS and ICANS were not reduced
  - CRS:
    - Any grade CRS: 93%
    - High-grade CRS: 7%
  - ICANS:
    - Any grade ICANS: 66%
    - High-grade ICANS: 40%
- Differential pattern of cytokine elaboration in patients with breakthrough CRS

Frigault et al in press

Prophylactic itacitinib decreases IEC-associated CRS and ICANS







- Part 1 Safety Lead In (n=63)
  - Grade 2+ CRS 22%
  - Grade 3+ CRS 2%
  - Duration of CRS 5d (1-11)
  - Grade 2+ ICANS 19%
  - Grade 3+ ICANS 13%
  - Duration of ICANS 3d (1-17)
- Part 2 Randomized Study (n=48)
  - Grade 2+ CRS 17% v 57%
  - Grade 3+ CRS 4.3% v 13.0%
  - Duration of CRS: 5d (3-12) v 4d (1-8)
  - Grade 2+ ICANS 9% v 22%
  - Grade 3+ ICANS 4% v 13%
  - Duration of ICANS 2d (2-11)

Frigault et al Blood

※イエスカルタの添付文書の効能・効果を参

CAR T-cell Toxicity Mitigation and Prevention: Open Questions

Is there a role for ppx anakinra or therapeutic use outside of IEC-HS?

Should everyone get ppx steroids?

Are there better ways of targeting the myeloid compartment?

Smarter CARs could provide answers but how to test them in a concentrated space with FDA approved products is a challenge.

演者作成

Summary: Patient Selection and Optimization for CAR T-cells

- CAR T-cells remain the optimal curative option for primary refractory/early relapsing LBCL in the 2L and for later relapsing patients in the 2L (if transplant ineligible) and in the 3L over all other available (and expanding) therapies

- Although several models exist to determine prognosis with CAR, none identify a group of patients with <25% long-term PFS and so this remains the best option for even the high-risk patients.

- Optimal bridging and cytokine therapy may be the key to improving efficacy and safety options for these high-risk patients.

- Early and expedited referral of all eligible patients is essential to reaching more patients and optimizing outcomes