Development and Optimization of Dual Targeted LV20.19 CAR T cells for B-cell non-Hodgkin Lymphoma

Nirav Shah, MD MS
Professor of Medicine
Division of Hematology & Oncology
Medical College of Wisconsin

knowledge changing life

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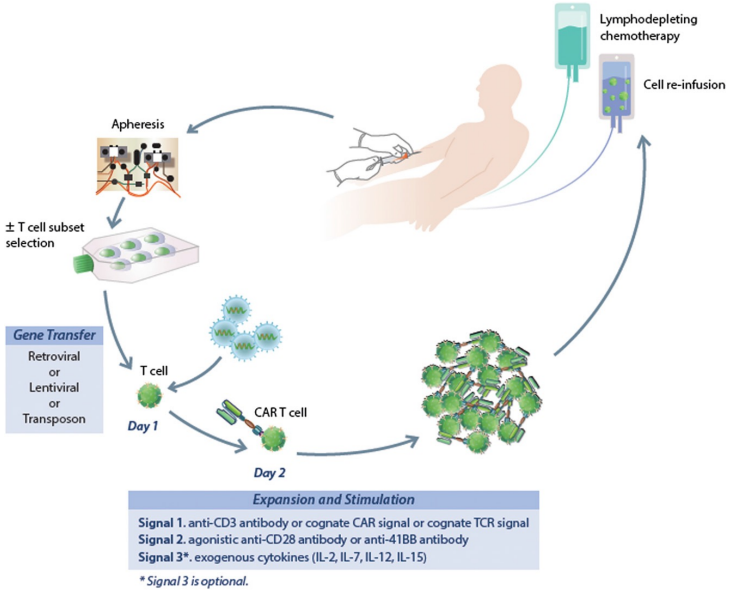
Disclosure Summary

Role	Relationship	Company/ies
Advisory Board	Advisor	Gilead-Kite, BMS-Juno, Miltenyi Biomedicine, Lilly Oncology, Incyte, Abbvie, Cargo, Beigene, Kite, Allogene, Astrazeneca, Genentech, Ipsen, and Galapagos
Research Funding	Researcher	Miltenyi Biotec, Lilly Oncology, Genentech
Scientific Advisory Board	Founder	Tundra Therapeutics

CAR-T Technology

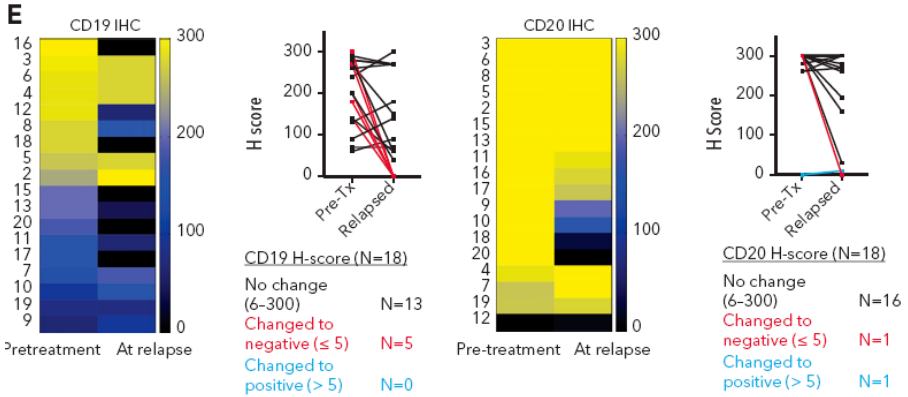
- Chimeric Antigen Receptor Modified T-cells
- Utilizes a viral vector to insert a gene into T-cells to target malignant conditions with co-stimulatory signaling domains, generally CD3ζ with either 4-1BB or CD28
- Multiple anti-CD19 CAR-T cell products are FDA approved for B-cell NHL and B-cell ALL
- Despite high ORR, relapse and deliver of CAR-T remain significant burdens



Mato A, Porter DL. *Blood. 2015.*

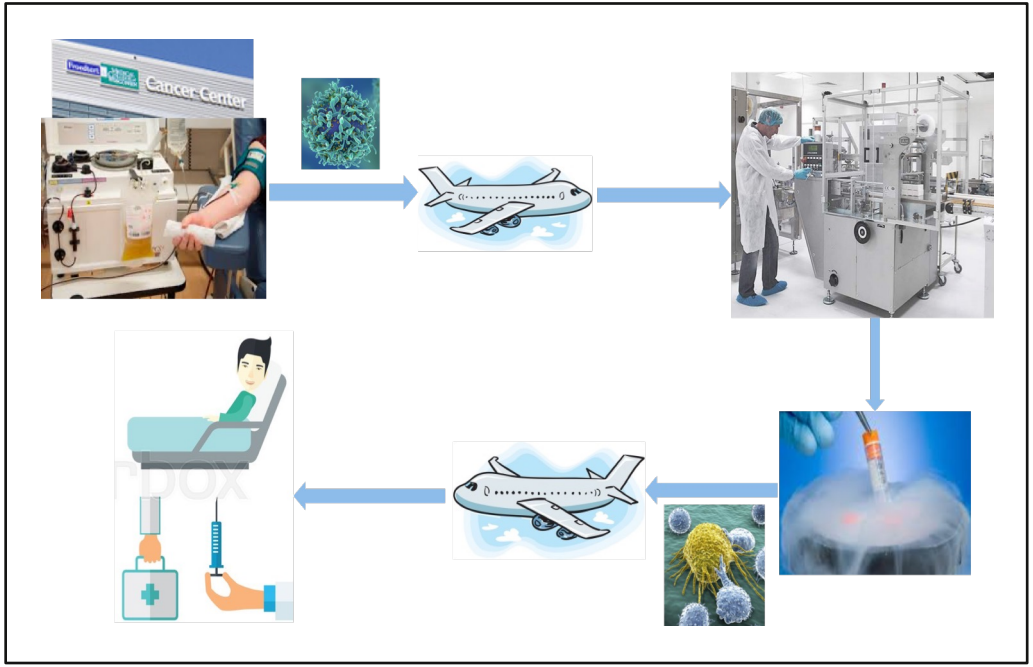
Limitation #1: CD19 downregulation

- Multiple forms of CD19 CAR T-cell therapy are approved for relapsed DLBCL with a long-term PFS ranging 30-40%
- CD19 loss is found in approximately 30% of large B-cell lymphoma patients after CAR T-cell therapy
- Recent studies have demonstrated that CD20 expression is stable even among patients who have CD19 loss after anti-CD19 CAR failure
- These data suggest dual targeting may be effective in improving CAR outcomes



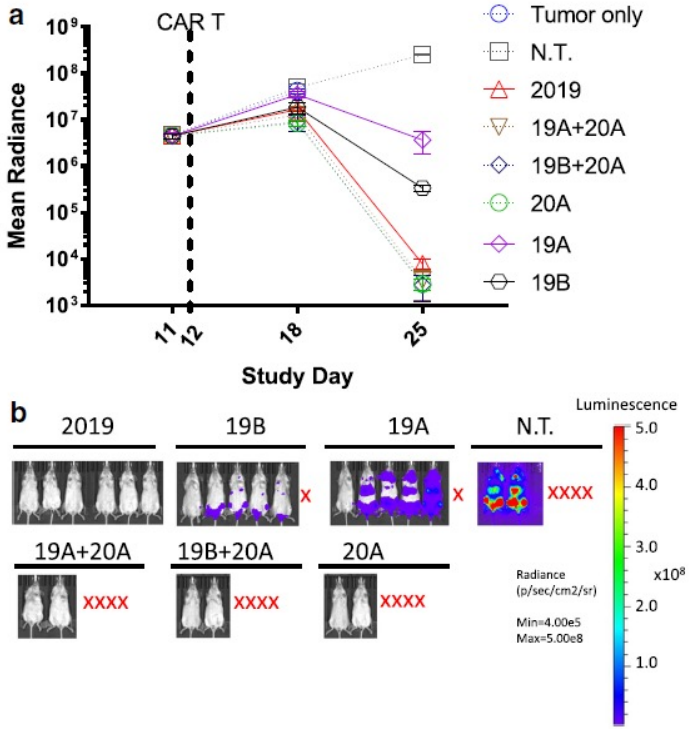
Limitations #2: Manufacturing

- Third-party, off-site centralized manufacturing process can be length and not feasible for patients with rapidly progressive disease.
- CAR-T cells are cryopreserved on delivery which may impact actual number of viable cells delivered to patients.
- Real world studies demonstrate 8% of patients who underwent apheresis never received CAR T-cell product



Nastoupil, L.J., M.D. *J Clin Oncol, 2020.* ***0(0): p. JCO1902104.***

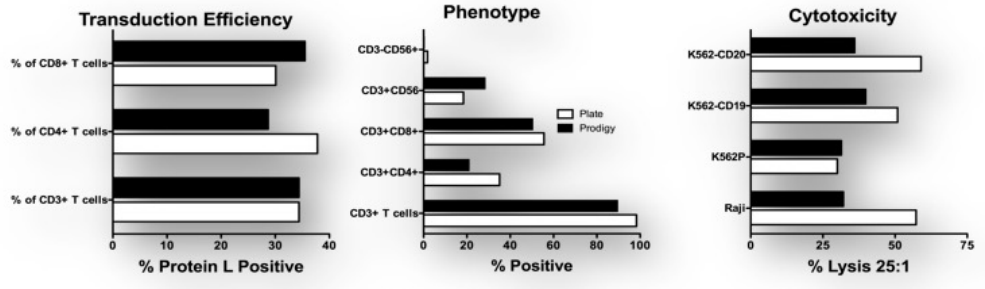
LV20.19 CAR T-cells



Schneider D et al. *Journal for Immunotherapy of Cancer. 2017*

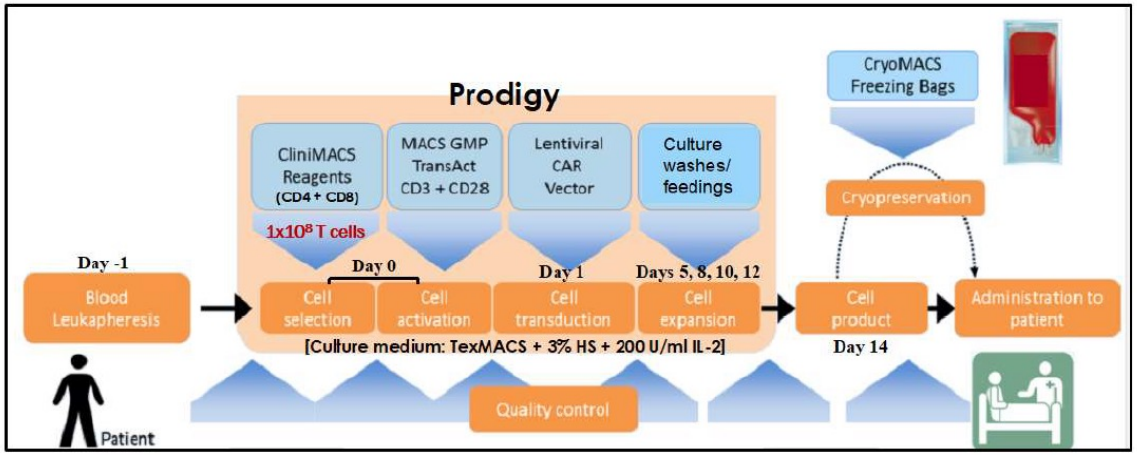
Left: high tumor burden mice treated with LV20.19 configuration all alive and in remission at day 25

Below: Prodigy manufactured LV20.19 CAR T-cells demonstrate cytotoxicity against K562 CD20 and CD19 cell lines with a well distributed CD4/CD8 phenotype



Zhu, F., N. Shah, et al. *Cytotherapy, 2018.* ***20(3): p. 394-406.***

Prodigy CliniMACS Manufacturing



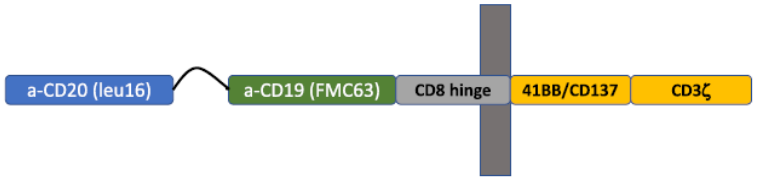
- CliniMACS Prodigy for point of care manufacturing
- 14-day fixed timeline
- Fresh in/Fresh out (goal)
- IL-2 for cell expansion
- In-process testing on Day 8 for sterility, endotoxin, transduction efficiency, mycoplasma, gram stain

Phase 1 Clinical Trial

- Completed a Phase 1, first-in-human, dose-escalation study of LV20.19 CAR T-cells in patients with relapsed, refractory B-cell NHL or CLL
- 22 evaluable patients
  - 11 DLBCL, 7 MCL, 3 CLL, 1 FL
- LDP: Flu 30mg/m^2 x 3 days + Cytoxan 500 mg/m^2 x 1 day

nature medicine
LETTERS
https://doi.org/10.1038/s41591-020-1081-3
Check for updates

Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial



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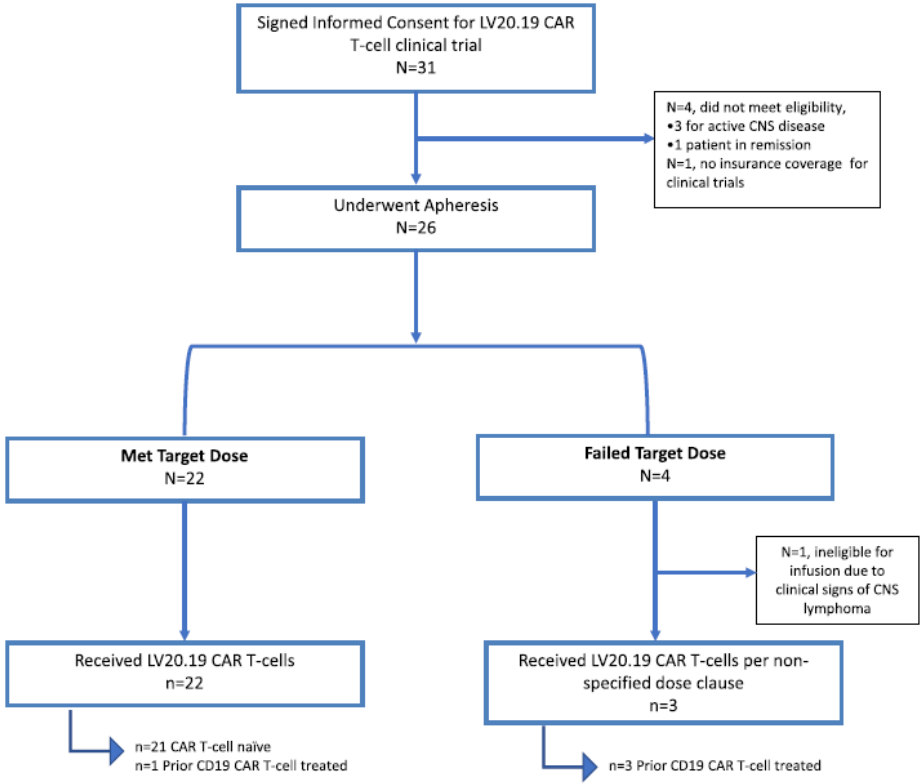
Trial Design



- Dose escalation with 3 dose levels
  - $2.5x10^5$ cells/kg
  - $7.5x10^5$ cells/kg
  - $2.5x10^6$ cells/kg
  - No DLT's in first two cohorts
  - $2.5x10^6$ cells/kg chosen for expansion

Manufacturing Characteristics

Parameter (Mean ± SD) [a]	Apheresis Product	Day of Culture 0 [b]	Day of Culture 8	Day of Culture 14
% CD3 T-cells	41.8 ± 20.9%	85.5 ± 9.6%	98.7 ± 1.6%	99.1 ± 1.3%
CD4:CD8 ratio (total T-cells)	1.6 ± 1.7	1.8 ± 1.6	3.5 ± 3.5 **	2.0 ± 2.2
CD4:CD8 ratio (CAR T-cells)	NA	1.8 ± 1.6 *	5.1 ± 5.6	3.1 ± 2.7
# CD3 T-cells	NA	1.00e8 ± 0	1.16e9 ± 0.47e9	3.33e9 ± 0.91e9
Fold total CD3 Expansion	NA	NA	11.7 ± 4.7	33.3 ± 9.1
% T-cells Transduced	NA	NA	14.4 ± 6.0%	17.4 ± 5.3%
# CAR T-cells	NA	NA	1.81e8 ± 1.26e8	5.76e8 ± 2.42e8
% Viability (trypan blue)	99.7 ± 0.5%	97.8 ± 2.8%	95.5 ± 2.6%	93.4 ± 2.7%

- Majority of patients would have reached goal dose by Day 8 of manufacturing
- Viability was high at harvest on average >90%
- No manufacturing failures in CAR naïve patients

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Safety: CRS/Neurotoxicity

Adverse Events Grade 3 or Higher (2.5x10^6 cells/kg n=16)	All Grades n (%)	Grade≥3 n (%)
Cytokine Release Syndrome[1]	12 (75)	1 (6)
Neurotoxicity[2]	5 (31)	3 (19)
Lymphocyte count decreased	14 (88)	14 (88)
Neutrophil count decreased	13 (81)	11 (69)
White blood cell decreased	14 (88)	12 (75)
Anemia	12 (75)	7 (44)
Platelet count decreased	9 (56)	5 (31)
Hypoxia	4 (25)	3 (19)
Hypocalcemia	10 (63)	1 (6)
Hematuria	4 (25)	1 (6)
Hyperglycemia	4 (25)	1 (6)
Lymphocyte count increased	2 (13)	1 (6)
Alanine aminotransferase increased	6 (38)	1 (6)
Creatinine increased	7 (44)	1 (6)

Focused on patients receiving 2.5x10^6 cells/kg (n=16) to be used in this trial

- 1 DLT: Grade 4 CRS: high tumor burden DLBCL, required dialysis for AKI due to TLS and Grade 4 neurotoxicity requiring intubation (only patient requiring ICU level care)
- 2 other Grade 3 NTX responded to steroids or IT chemotherapy
- 7 patients required tocilizumab for management

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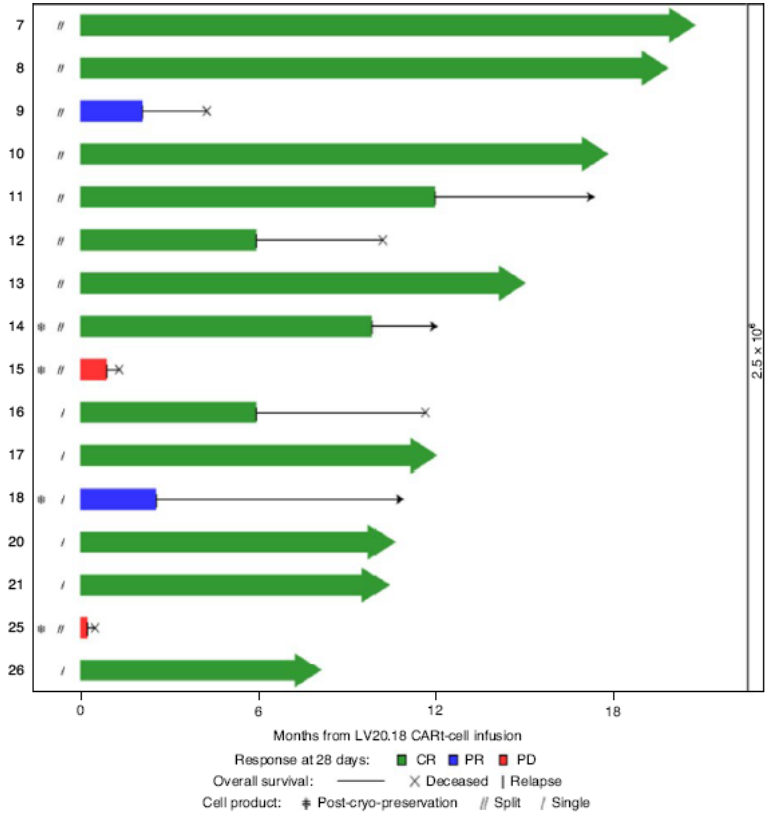
Response

Clinical outcomes at day 28	
Day 28: ORR, all dose levels ($n=22$)	18 (82%)
CR	14 (64%)
PR	4 (18%)
Day 28: ORR, dose of 2.5×10^6 cells per kg ($n=16$)	14 (88%)
CR	12 (75%)
PR	2 (13%)
Day 28 ORR, dose of 2.5×10^6 cells per kg, fresh infusion ($n=12$)	12 (100%)
CR	11 (92%)
PR	1 (8%)
DLBCL day 28 ORR ($n=11$)	10 (91%)
CR	7 (64%)
PR	3 (27%)



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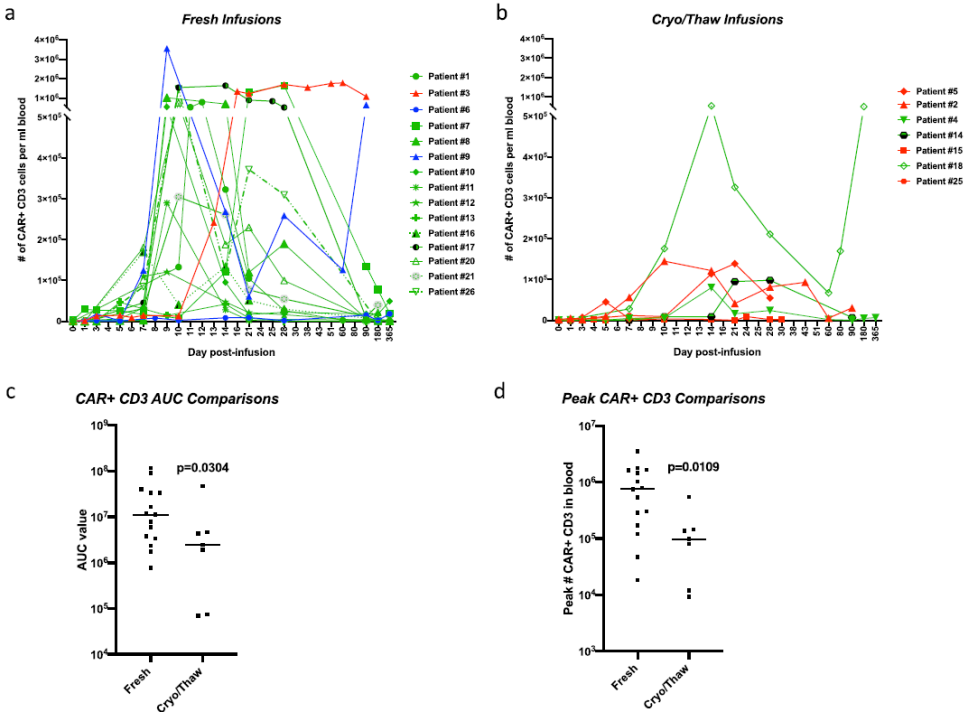
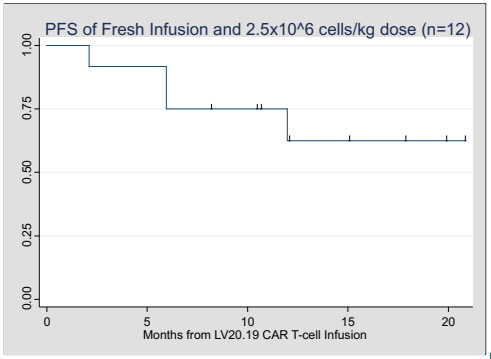
Fresh versus Post-Cryopreserved LV20.19 infusion



- 15 patients received fresh infusion
- ORR 93% vs 57% with fresh infused cells
- In vivo peak expansion and AUC for LV20.19 CAR favored fresh infusion



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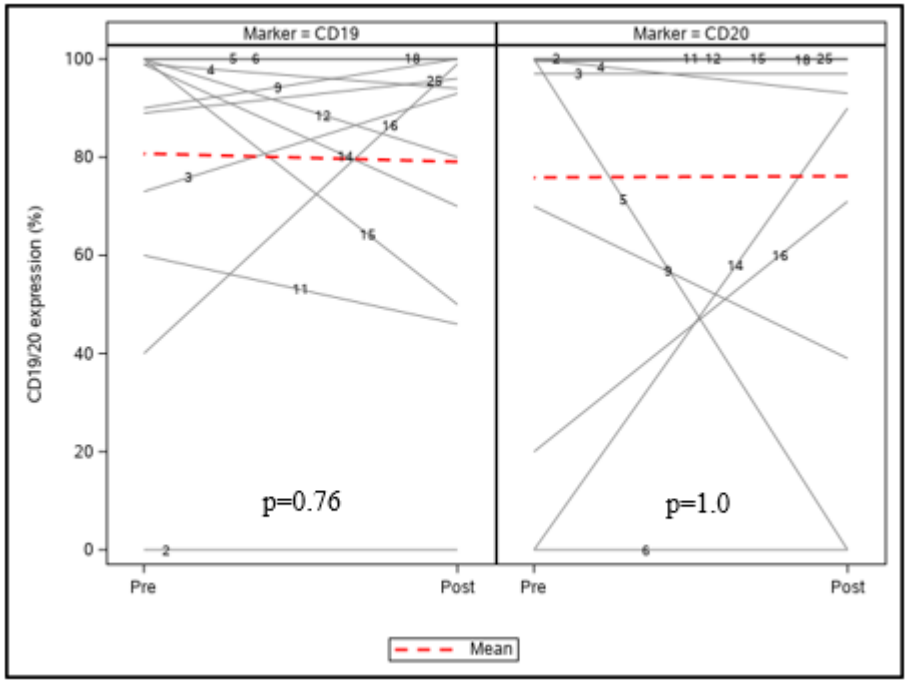
Treatment Failure

Despite high ORR and CR rates, relapse did occur

- Cell dose impacted relapse
- Relapsing patients retained CD19/CD20 expression suggesting our that dual targeting may mitigate CD19 negative relapse

Subjects with Disease Progression													
Subject #	**02***	**03**	**04**	**05***	**06**	**09**	**11**	**12**	**14***	**15***	**16**	**18***	**25***
Disease	DLBCL (MYC+)	DLBCL (MYC+)	MCL	MCL[4]	CLL	DLBCL (Richters)	DLBCL (MYC+)	MCL	FL[5]	MCL	DLBCL (MYC+)	DLBCL	MCL
Cell Dose (cells/kg)	**2.5x10^5**	**2.5x10^5**	**7.5x10^5**	**7.5x10^5**	**7.5x10^5**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**	**2.5x10^6**
Day 28 Response	PR	PD	CR	PD	PR	PR	CR	CR	CR	PD	CR	PR	PD
Time of Progression post-CAR	3 mon	N/A	12 mon	N/A	3 mon	2 mon	12 mon	6 mon	10 mon	N/A	6 mon	2 mon	N/A
CD20 status pre LV20.19	100%[1] 100%[2]	97%[2] 1990.24[3]	100%[2]	100%[2,4]	Neg[1]	70%[1]	100%[1] 100%[2]	100%[1]	Neg[1] Neg[2]	100%[2]	20%[1]	100%[1] 99.5%[2]	100%[2] 12843.5[3]
CD19 status pre LV20.19	Neg[1] Neg[2]	73%[2] 3064.86[3]	90%[1] 99%[2]	100%[2,4]	100%[1]	90%[1]	30%[1] 60%[2]	100%[1]	100%[1] 99%[2]	100%[2]	40%[1]	70%[1] 100%[2]	89%[2] 2987.58[3]
CD20 status progression	100%[1]	100%[1] 97%[2] 4892.56[3]	93%[2]	Neg[1,2] 42.62[3]	Neg[2] 157.82[3]	39%[2] 1571.55[3]	100%[1] 100%[2] 17912.66[3]	100%[1]	90%[1]	100%[1] 100%[2] 4704.99[3]	71%[2] 3995.53[3]	100%[1]	100%[2] 34929.6[3]
CD19 status progression	Neg[1]	100%[1] 93%[2] 10901.15[3]	94%[2]	100%[2] 9675.35[3]	100%[2] 5512.6[3]	100%[2] 12254.76[3]	35%[1] 46%[2] 1779.21[3]	80%[1]	70%[1]	95%[1] 50%[2] 4583.81[3]	99%[2] 2031.15[3]	100%[1]	96%[2] 4286.52[3]

CD19 & CD20 Expression



- No correlation between CD19 and CD20 expression at baseline and relapse among treatment failure patients

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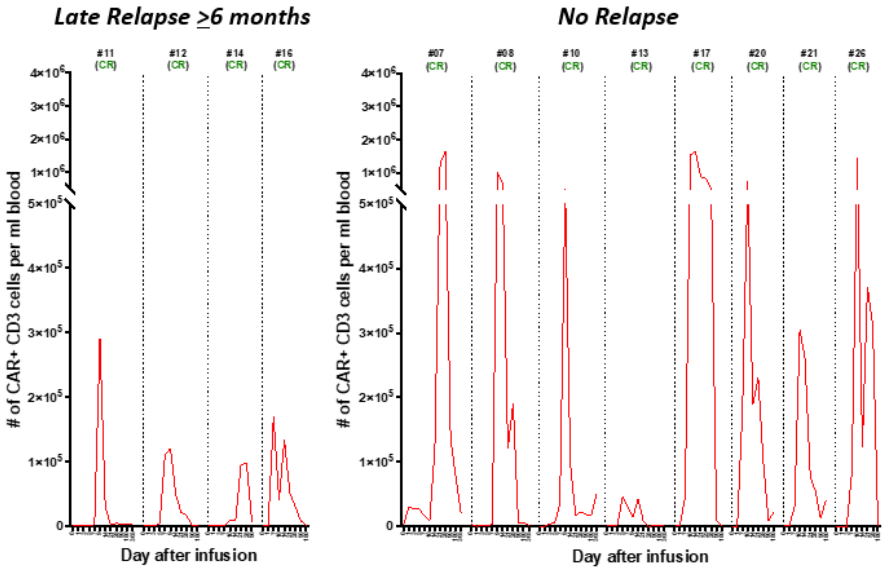
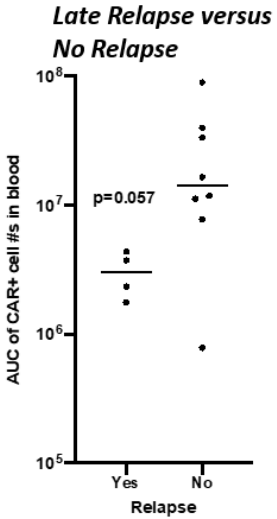
Late Relapse





Several patients had late relapse >6 months after CAR infusion. This correlated with poor in-vivo peak expansion.

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Key Takeaways

1) LV20.19 CAR T-cells have promising safety and efficacy at a dose of $2.5x10^6$ cells/kg.
2) Patients receiving “fresh” or non-cryopreserved CAR T-cells had improved expansion kinetics and higher ORR (limited # of patient)
3) Relapse/Progression was NOT associated with changes in CD19/CD20 expression
4) Late relapse correlated with poor in-vivo expansion within the first 28 days
5) Proof of concept: CAR-T cells can be manufactured in a POC fashion and safely/reliably delivered to patients

Lessons Learned

Managing Neurotoxicity

- Lessons Learned
  - First severe toxicity was patient on LV20.19 CAR T-cell trial with Grade 3 Neurotoxicity/ICANS
  - 71-year-old male with high tumor burden DLBCL
  - Treated with high dose dexamethasone, Pulse Solumedrol with no improvement
  - Steroid Refractory ICANS: CSF with elevated WBC—Should we treat the source with intrathecal chemotherapy??? Similar to other inflammatory conditions (e.g. neurotoxicity with HLH is managed with IT

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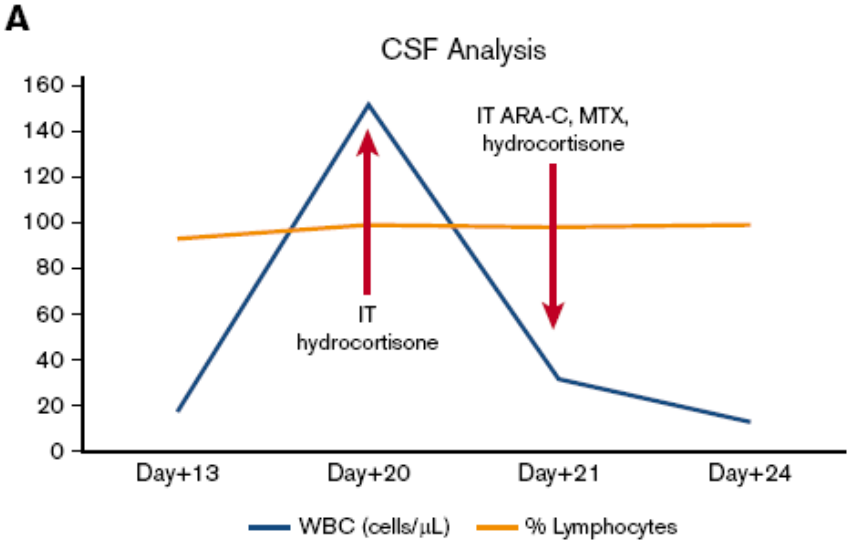
Using IT to Mitigate ICANS



- After IT hydrocortisone, improvement in alertness
- Then administered triple therapy (IT MTX/ARA-C/Hydrocortisone), following day awakens, answering questions, now oriented
- Started steroid taper, with rehab full resolution

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6 years post-CAR-T at our Annual BMT/CT Celebration of life

ICANS management

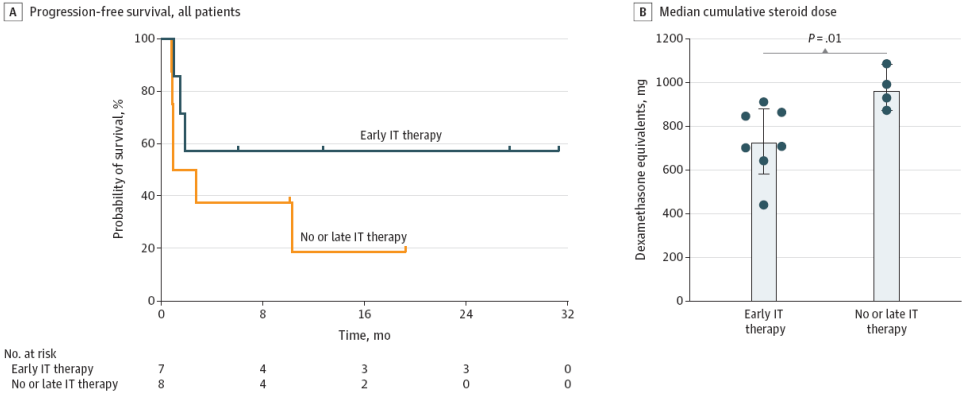
Building off this Experience:

- Started utilizing IT chemotherapy for high grade ICANS for CAR20.19 trial
- Retrospective review of all CAR patients with ICANS
- Found early IT therapy correlated with improves PFS and decreased steroid usage

Research Letter
March 10, 2022
JAMA Oncology

Use of Early Intrathecal Therapy to Manage High-Grade Immune Effector Cell-Associated Neurotoxicity Syndrome

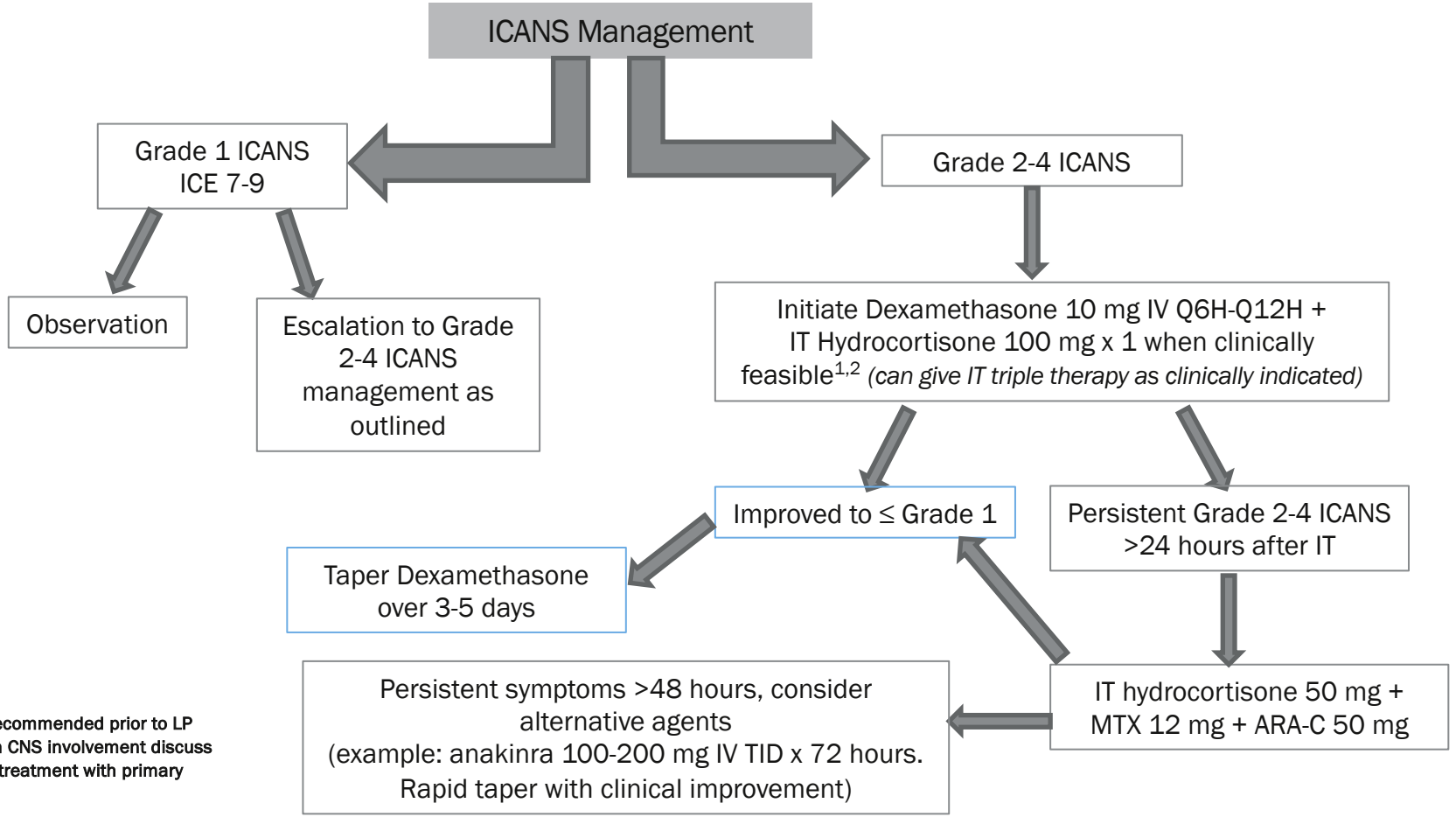
Joanna C. Zurko, MD[1]; Bryon D. Johnson, PhD[1]; Emilie Aschenbrenner, PharmD[1]; Timothy S. Fenske, MD[1]; Mehdi Hamadani, MD[1]; Parameswaran Hari, MD[1]; Nirav N. Shah, MD, MS[1]



More on IT at ASH

- Multicenter analysis of IT chemotherapy for ICANS being presented by MCW Heme/Onc Fellow Adam Kidwell

Details:
Session Name: 906. Outcomes Research: Lymphoid Malignancies Excluding Plasma Cell Disorders: Poster III
Session Date: December 8, 2025
Session Time: 6:00 PM - 8:00 PM
Presentation Time: 6:00 PM - 8:00 PM
Room: OCCC - West Halls B3-B4

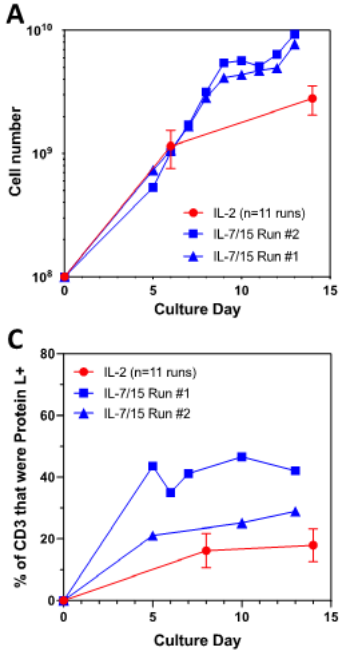


[1]CNS imaging recommended prior to LP
[2]In patients with CNS involvement discuss pros/cons of IT treatment with primary physician

Optimizing LV20.19 CAR T-cells

Optimizing CAR20.19 T-cells

- Can we do better?
  - Most patients achieved CAR T-cell dose by day 8 of manufacturing
  - Could extending time in culture impact CAR T-cell immunophenotype and lead to administration of a more “exhausted” CAR product?
  - Can “cooking the cells” in different cytokines produce a more robust product?

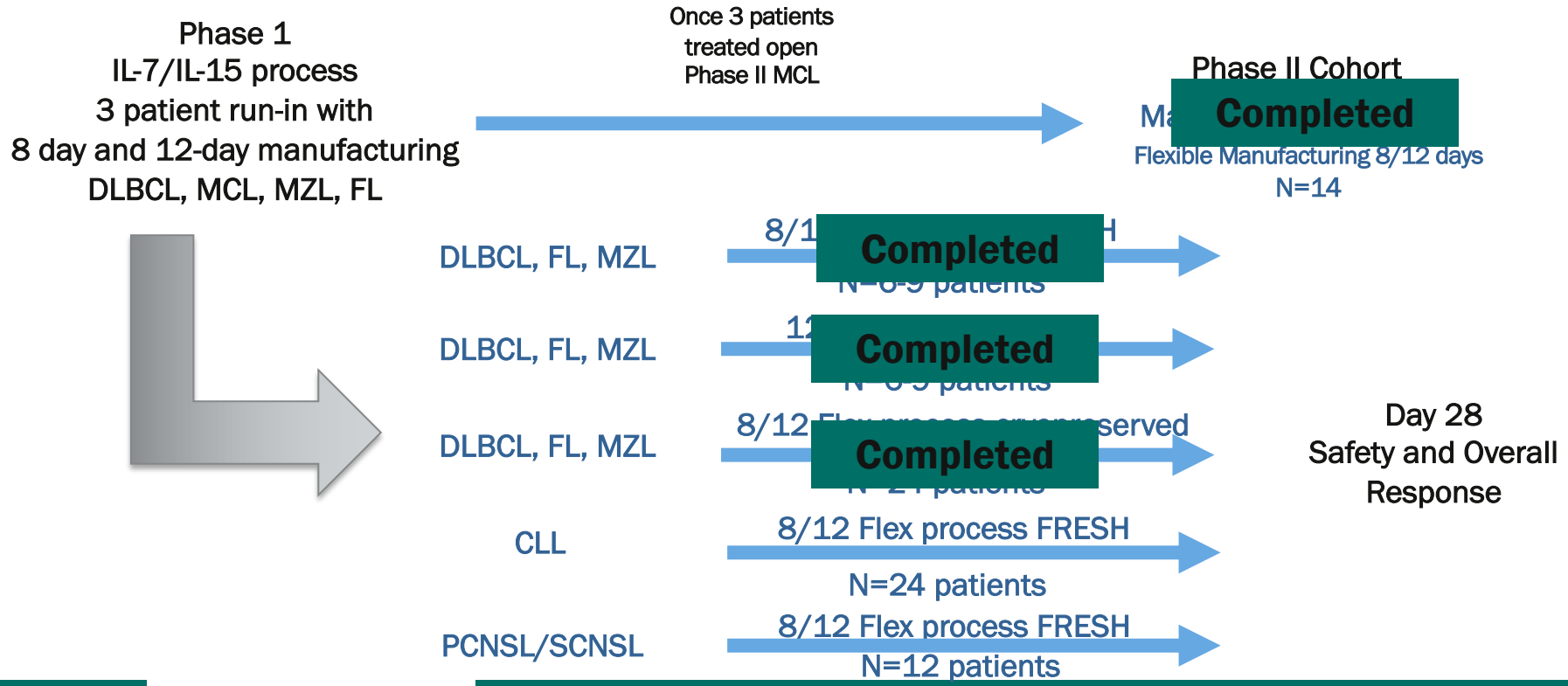


NEW TRIAL

- LV20.19 CAR T-cells expanded with IL-7 and IL-15 (to improve phenotype and persistence) manufactured at varying length of time (8 versus 12 days)
- Phase II ARM in MCL to assess efficacy in disease specific manner
- Evaluate importance of cryopreservation on clinical outcomes

Xu et al. Blood 2014 and Zhou et al. Protein and Cell 2019

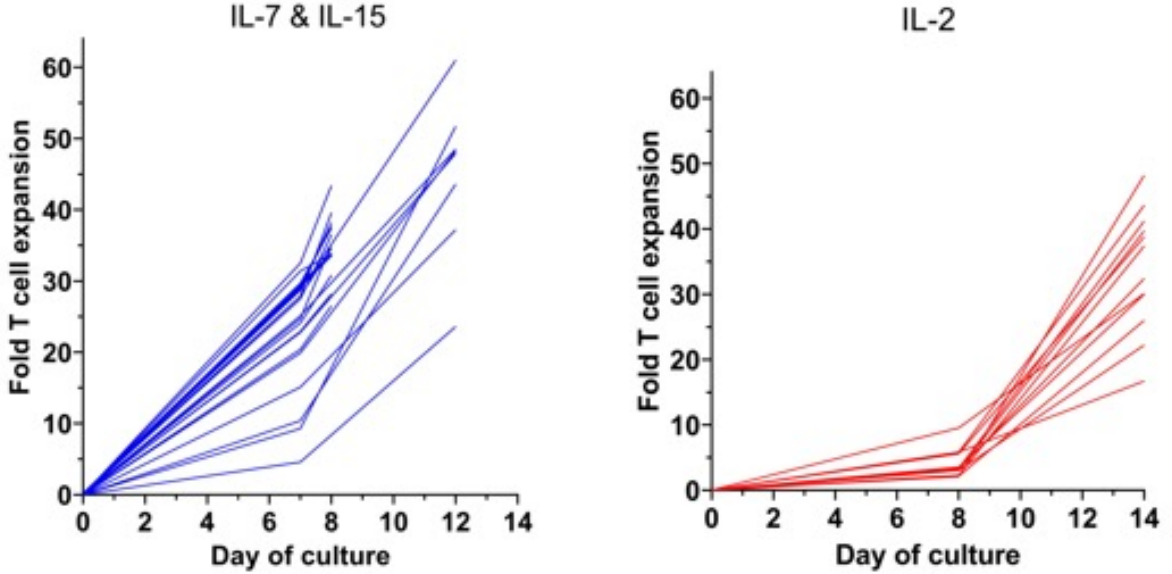
IIT LV20.19 CAR T-cells in B-cell NHL



Manufacturing Data (IL-7/IL-15 vs IL-2)

Improved T-cell expansion in-vitro with IL-7 and IL-15

Figure 1:Fold T-cell expansion in IL-7 & IL-15 vs IL-2



Manufacturing Data (IL-7/IL-15 vs IL-2)

Table 1:Manufacturing results of LV20.19 CAR T-cells expanded in IL-7 & IL-15 vs IL-2

	IL-7 and IL-15 manufacturing (n=21)					IL-2 manufacturing (n=12)			
Parameter (Mean ± SD)[a]	Apheresis Product (n=21)	Day 0[b] (n=21)	Day 7 In-Process (n=21)	Day 8 Harvest[c] (n= 13)	Day 12 Harvest[c] (n= 8)	Apheresis Product (n=12)	Day 0[b] (n=12)	Day 8 In-process (n=12)	Day 14 Harvest (n=12)
% CD3 T Cells	37.6 ± 20.9%	91.0 ± 4.7%	99.3 ± 0.5%	99.5 ± 0.2%	99.2 ± 0.9%	42.1 ± 22.6%	82.4 ± 9.9%	98.3 ± 1.8%	99.0 ± 1.0%
CD4:CD8 Ratio (Total T Cells)	1.6 ± 1.4	2.3 ± 2.1	2.2 ± 2.2	3.3 ± 2.8	1.7 ± 1.8	1.9 ± 2.1	2.0 ± 1.9	3.4 ± 4.2	2.0 ± 2.8
CD4:CD8 Ratio (CAR+ T Cells)	N/A	N/A	4.7 ± 4.0	5.5 ± 5.1	3.1 ± 2.7	N/A	N/A	5.5 ± 7.4	3.2 ± 3.5
# CD3 T Cells	N/A	9.10 ± 0.47e7	2.12 ± 0.73e9	3.20 ± 0.43e9	4.07 ± 0.96e9	N/A	1.00 ± 0e8	1.02 ± 0.56e9	3.39 ± 0.39e9
Fold CD3 Expansion	N/A	N/A	23.4 ± 7.7	35.0 ± 4.6	45.2 ± 11.3	N/A	N/A	10.4 ± 5.7	33.9 ± 9.3
% CAR+ T Cells	N/A	N/A	22.2 ± 8.7%	26.3 ± 6.2%	19.6 ± 9.0	N/A	N/A	13.1 ± 5.6%	16.6 ± 5.5%
# CAR+ T Cells	N/A	N/A	5.19 ± 2.94e8	8.52 ± 2.64e8	8.09 ± 4.45e8	N/A	N/A	1.51 ± 1.48e8	5.81 ± 3.06e8
% Viability (Trypan Blue)	99.9 ± 0.4%	98.4 ± 1.2%	95.7 ± 3.4%	98.3 ± 0.9%	96.2 ± 2.4%	99.9 ± 0.3%	98.2 ± 1.6%	94.4 ± 2.9%	93.5 ± 3.0%

a- All data is expressed as mean ± standard deviation; b- Day 0 represents cells immediately after CD4/CD8 enrichment prior to culture; c- Patients received CAR T-cells either at day 8 or 12 of manufacturing on IL7+15 protocol

IL7+15 Produces a More Robust CAR Product than IL2

Figure 1: Polyfunctionality of LV20.19 CAR T-cells expanded in IL-2 versus IL-7 & IL-15

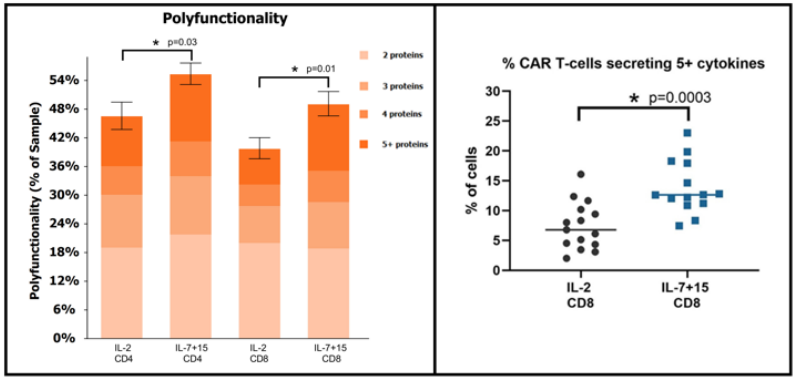
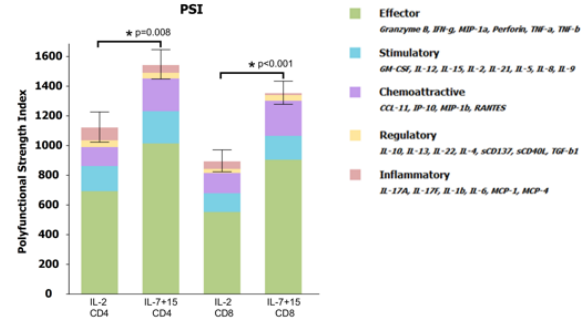


Figure 2: Polyfunctional strength index (PSI) of LV20.19 CAR T-cells expanded in IL-2 versus IL-7 & IL-15



<u>Single cell cytokine secretions via Isoplexis Device:</u>

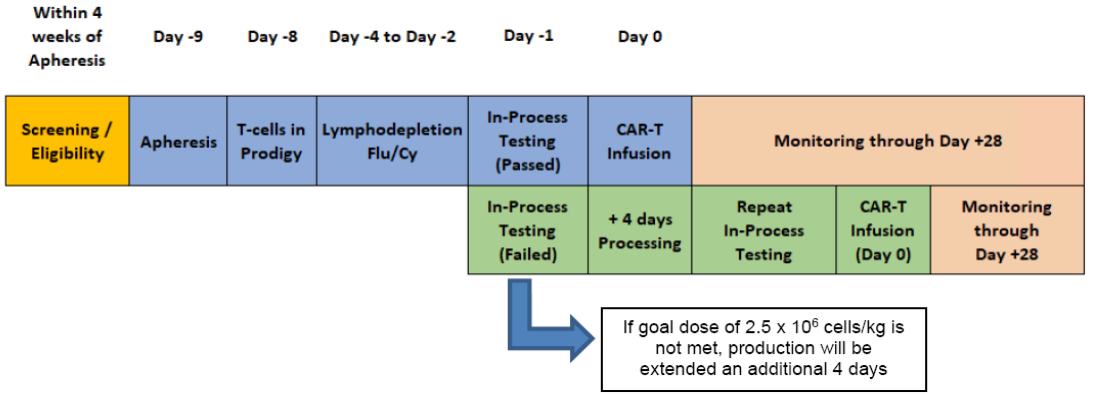
IL-7/IL-15 manufacturing results in CAR T cells with increased <u>polyfunctionality</u> (ie, ability to secrete 2 or more cytokines), improved PSI in CD4 and CD8, and in % of CAR-T cells secreting 5+ cytokines

8/12 flex versus 12-day fixed process

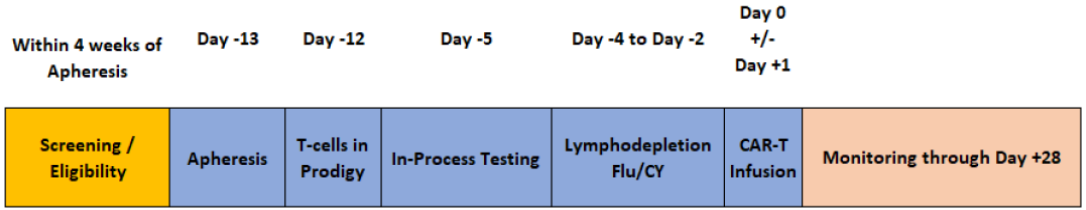
Patients on the 8/12 flexible arm had in-process testing on Day 7 of MF, if the target dose was not met, they could flex to a 12-day process to allow additional culture time

LDP- Fludarabine 30 mg/m2 and Cyclophosphamide 300 mg/m2 Day -4, -3, -2

8/12 Flexible Process



Fixed 12-day Process

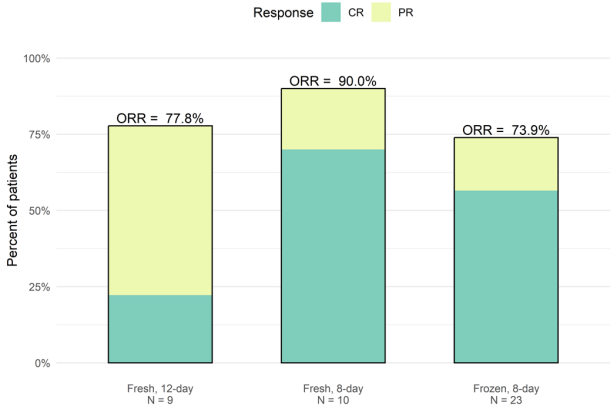


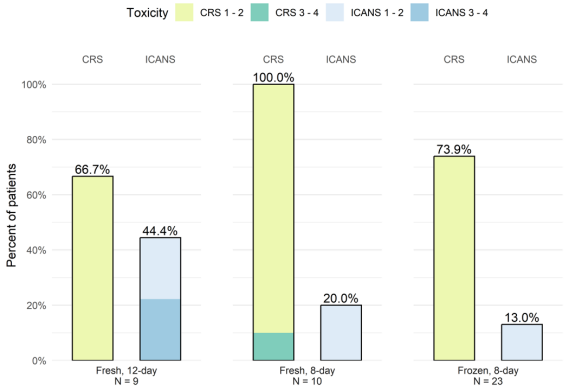
Patient Demographics

- Compared three arms: Fresh-8-day, Fresh-12 day, and Frozen 8-day
- All patients had a diagnosis of CAR naïve, relapsed, refractory DLBCL or FL
- MF was successful in 100% of regardless of arm and all patients on the flexible 8/12 process achieved target dose of 2.5x10e6 at Day 8.

Table 1: Demographics N=42	Fresh, 8-day N=10	Fresh, 12-day N=9	Frozen, 8-Day N=23
Age	67 (44 - 78)	67 (61 - 73)	66 (45 - 78)
Male Sex	6 (60%)	8 (89%)	15 (65%)
Elevated LDH	7 (70%)	8 (89%)	15 (65%)
8-day MF	10 (100%)	0 (0%)	22 (96%)
Fresh Infusion	9 (90%)	7 (78%)	0 (0%)

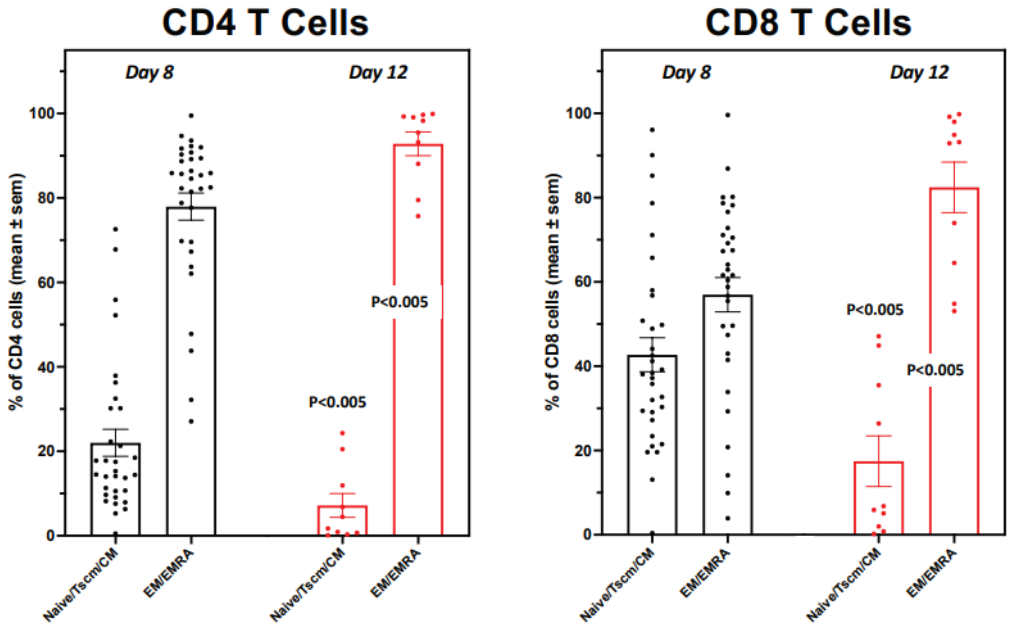
Clinical Outcomes





- The ORR/CR for 8-day fresh vs 12-day fresh was 90%/70% vs 78%/22%, p=0.58 (ORR), and for 8-day fresh vs 8-day frozen was 90%/70% vs 74%/57%, p=0.40 (ORR).
- Trend towards more frequent CRS in 8-day fresh products vs 8-day frozen (100% vs 74%, p=0.09).

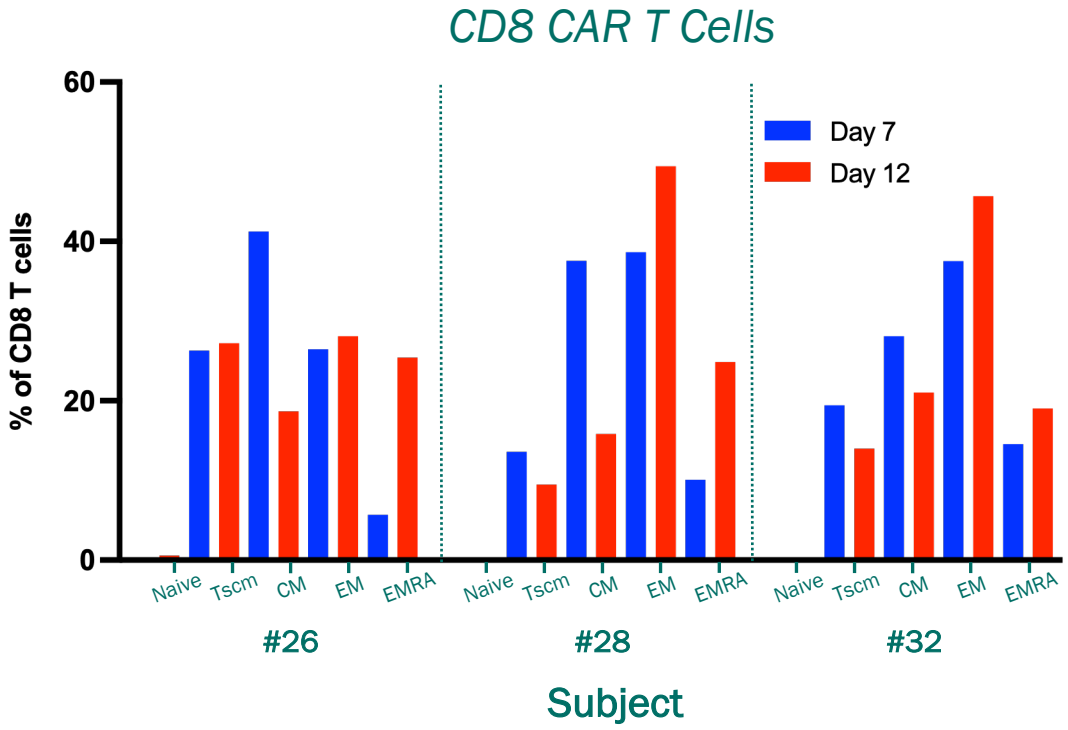
Immunophenotype 8 vs 12 days



CD4 (left panel) and CD8 (right panel) CAR T cells in final 8-day (n=32) and 12-day (n=10) manufactured LV20.19 cell products were analyzed by flow cytometry. Less differentiated T cell phenotypes (naïve, Tscm and CM) and more differentiated phenotypes (EM and EMRA) were separately grouped together. Both CD4 and CD8 CAR T cells in 8-day final products had a significantly less differentiated phenotype ($p<0.005$) than in 12-day products.

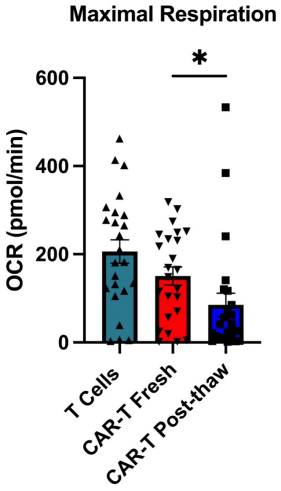
Intra-patient Immunophenotype

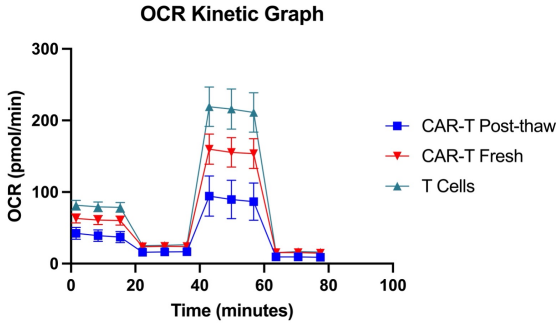
Intra-patient immunophenotyping of 3 pts assigned to the 12-day MF arm demonstrated a similar shift with decreasing CM T-cells and increasing EMRA T-cells between day 7 (in-process testing) and day 12 (harvest).

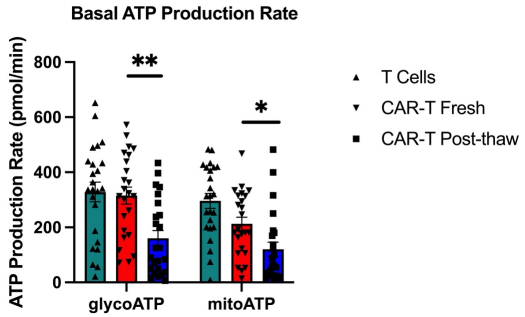


Impact of Cryopreservation on Metabolism

To assess whether cryopreservation affected the activation or bioenergetic capacity of administered CAR-T cells, we examined energy metabolism and activation status of T cells prior to CAR manufacturing, of CAR-T cells immediately before cryopreservation (“fresh”), and of CAR-T cells after thawing on the day of infusion (“post-thaw”) via Seahorse.



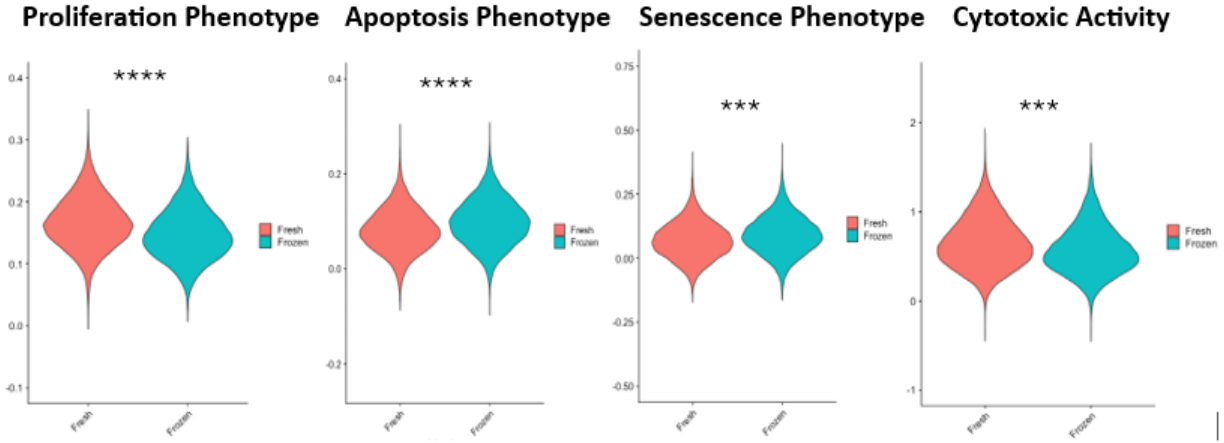




Statistically significant decreases in both the oxygen consumption rate (OCR), maximal respiratory capacity, ATP production in in the post-thaw CAR-T cells ($p<0.05$)

Transcriptional Changes with Cryopreservation

To characterize transcriptional changes induced by cryopreservation, we performed single-cell RNA sequencing on representative LV20.19 CAR-T products from 6 patients pre/post cryopreservation and thawing. **Genes indicative of apoptosis and senescence were elevated in cryopreserved CAR-Ts while proliferation and cytotoxicity markers were reduced (*p<0.05*) (Fig. 3).**



Conclusions

- IL-7/15 expanded, fresh CAR20.19 T cells manufactured in 8-days optimizes the product
- Shorter manufacturing >>> Cryopreservation

More at ASH 2025

Session Name: 711. Cell Collection and Manufacturing of HSPCs, CAR-T Cells, and Other Cellular Therapy Products: Poster I

Session Date: 12/6/2025

Presentation Time: 5:30:00 PM – 7:30:00 PM

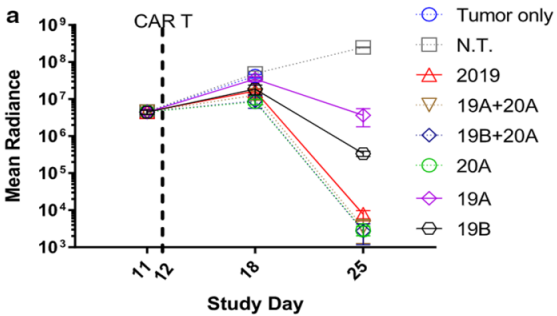
Location: OCCC - West Halls B3-B4

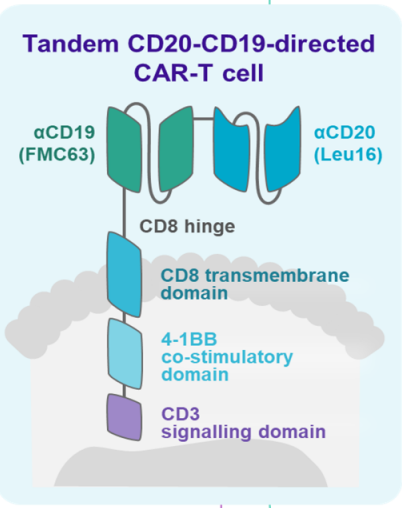
Expanding our work beyond MCW: Phase 1→multicenter Phase 2

Dual targeting with CD20-CD19 CAR-T cell may limit antigen escape

Antigen loss observed in ~ 30% of pts after CD19 CAR-T therapy[1]

Pre-clinical studies[2]





First-in-human trials

Phase I (NCT03019055)[3]

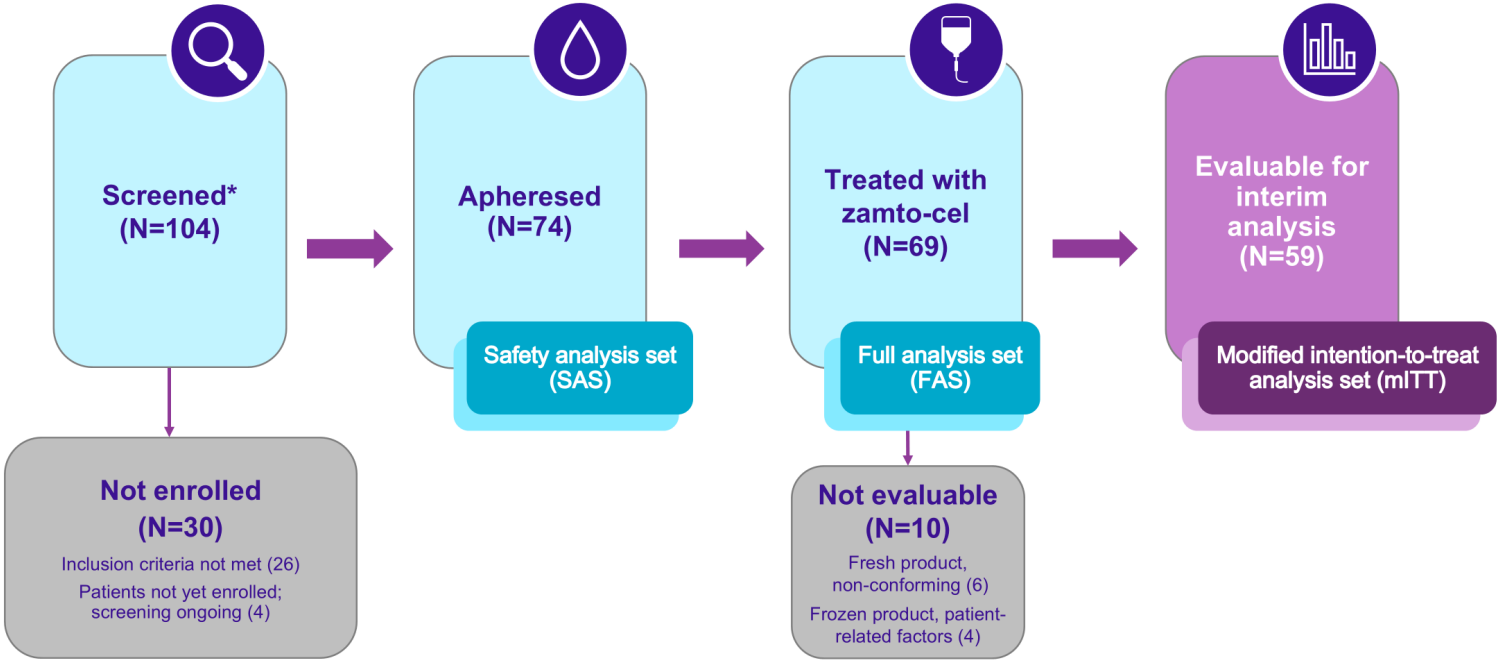
- Dose escalation and expansion trial
- Identified dose: 2.5x10e^6 cells/kg
- High Response Rate with durable remissions over >4 years post-treatment
- LTG 1497 CAR construct identical to MB2019.1 CAR construct

Phase I/II (DALY I, NCT03870495)[4]

- Multicenter, open-label
- Accessed feasibility, dosage, safety and toxicity
- Confirmed recommended dose of 2.5x10e^6 cells/kg
- Infused in 100% enrolled
- Well-tolerated, no CRS or neurotoxicity
- ORR at 75% with 5/12 achieving CR durable response

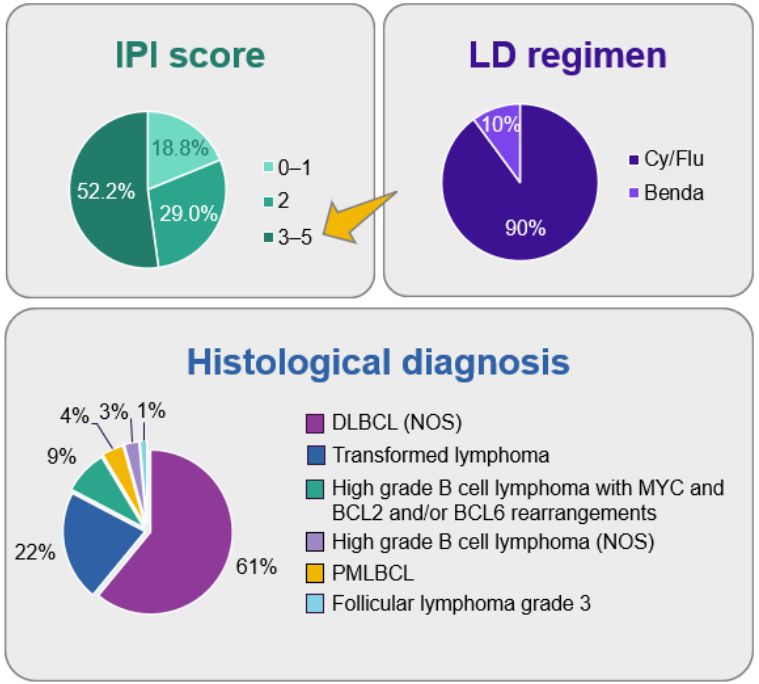
1. Majzner, R. and Mackall, C. Tumor Antigen Escape from CAR T–cell Therapy. Cancer Discov; 8(10); 1219–26;
2. Schneider D. et al. A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. J. ImmunoTherapy of (2017) 5:42;
3. Shah, N. N. et al. Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial. Nat Med 26, 1569-1575, doi:10.1038/s41591-020-1081-3 (2020)
4. Borchmann, P. et al. Phase I Trial of MB-CART2019.1 in patients with relapsed or refractory B cell non-hodgkin lymphoma: 2-year follow-up report. European Hematology Association Poster 1184 (2022).

Patient disposition:
59 patients were evaluated in the planned interim analysis (mITT)

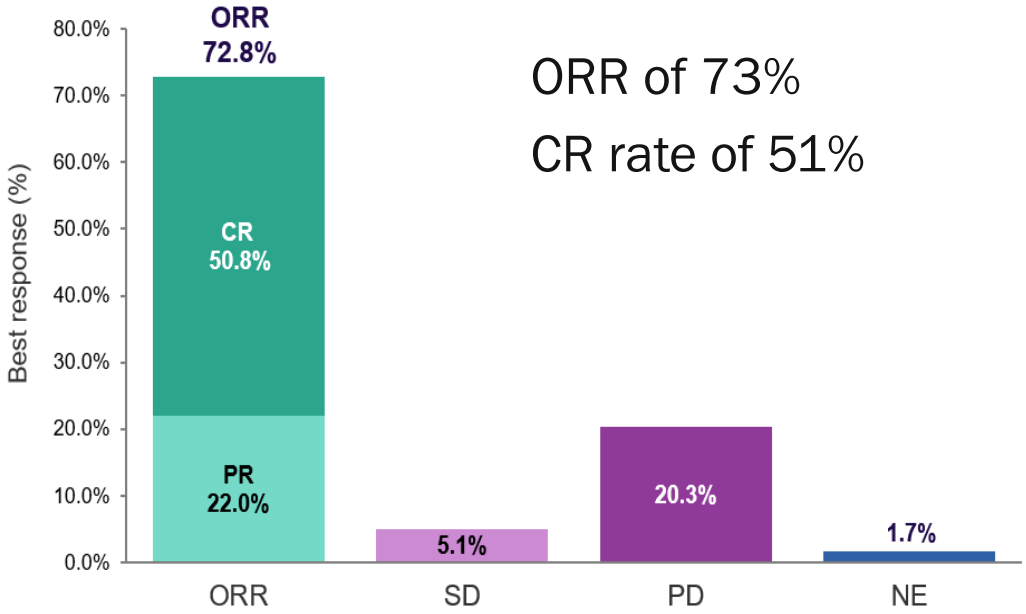


Patient Demographics

	FAS (N=69)
Median age, years (range)	65 (25–85)
Male sex, N (%)	47 (68.1)
Race, N (%)	
White	57 (82.6)
Asian	10 (14.5)
Black	1 (1.4)
Unknown	1 (1.4)
LDH elevated, N (%)	38 (55.1)
≥2 extranodal sites, N (%)	37 (53.6)*
Prior lines, N (%)	
2	52 (75.4)
3+	17 (24.6)
History of ASCT, N (%)	17 (24.6)
Bridging, N (%)	
Steroids	3 (4.3)
RT	1 (1.4)



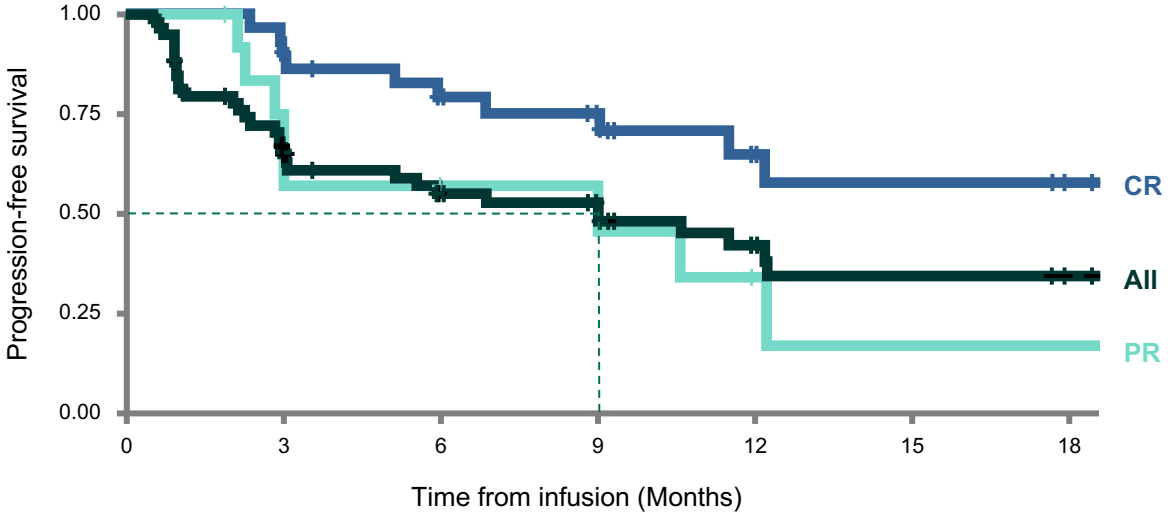
Response rates as Best Overall Response (BOR) showed high efficacy in the mITT (N=59) population



ORR of 73%

CR rate of 51%

Median Progression Free Survival=9 months



Number at risk

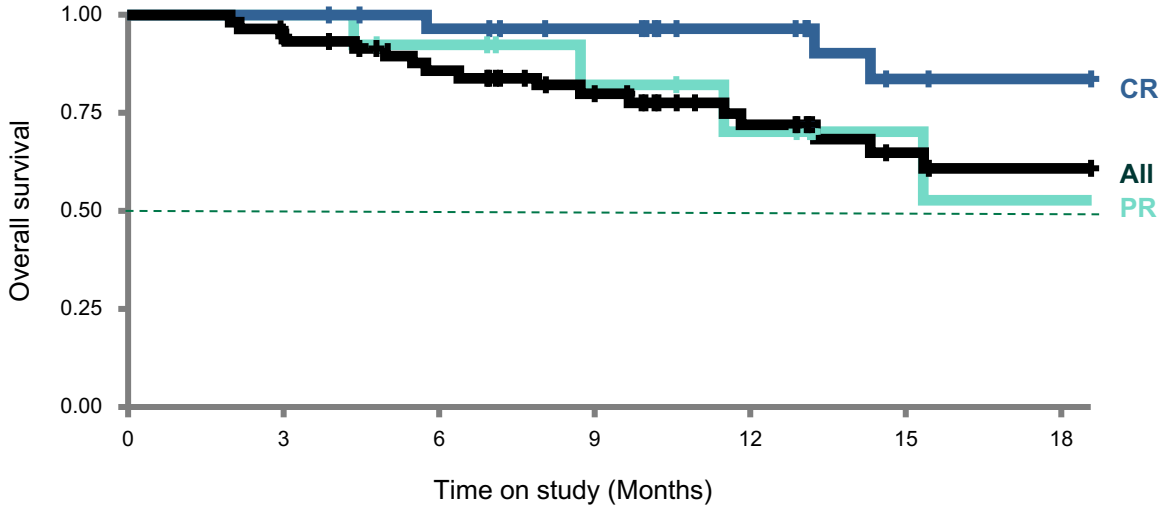
BOR								
	CR	30	26	21	17	10	8	6
	PR	13	7	5	5	2	1	1
	All	59	34	26	22	12	9	7

Progression-free Survival

- 6-month PFS: 55%

BOR, best overall response; CR, complete response; mFU, median follow-up; PFS, progression-free survival; PR, partial response.

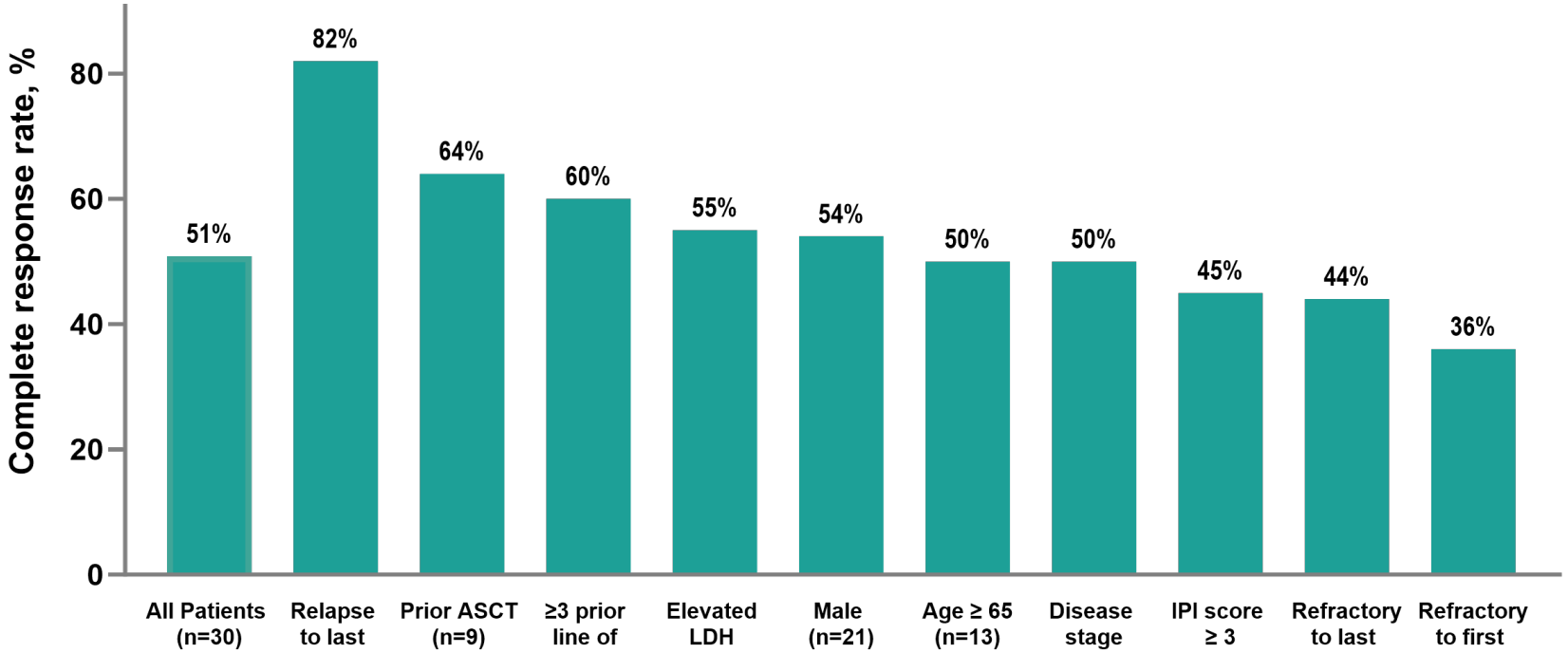
Median Overall Survival (OS) was not reached at the time of interim analysis



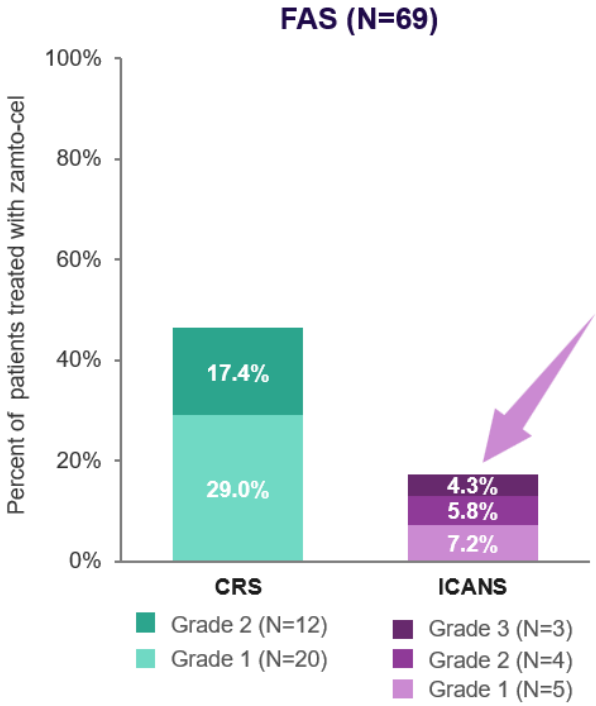
Number at risk

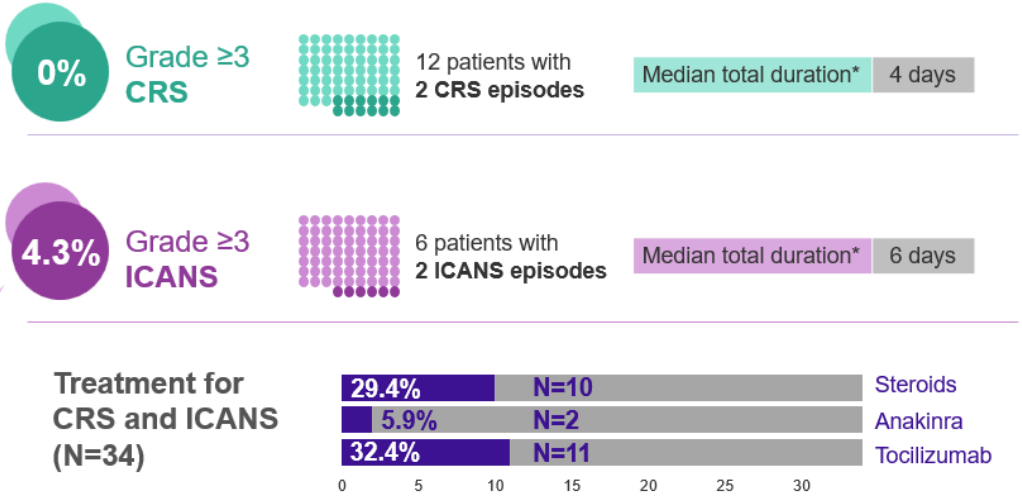
BOR								
	CR	30	30	27	24	18	12	11
	PR	13	13	11	8	6	4	3
	All	59	55	47	38	25	17	15

Zamto-cel: CRR as BOR by subgroups in mITT (N=59)



Low Incidence of CRS and ICANS – mostly low grade



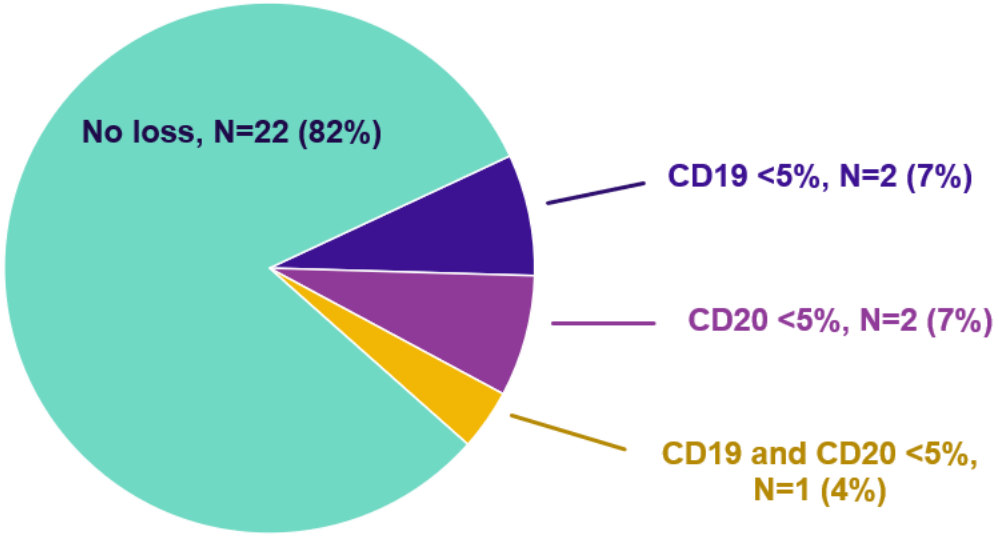


Adverse Events of Special Interest within 90 days

Toxicity*	Grade ≥3, N (%)**
Hematologic toxicities	43 (62.3)
Neutropenia/Neutrophil count decreased	33 (47.8)
Anemia/Hemoglobin decreased	14 (20.3)
Thrombocytopenia/Platelet count decreased	8 (11.6)
Infections	3 (4.3)
Deaths (due to any cause) ***	5 (7.2)
IEC-HS	1 (1.4)
Secondary malignancies	0

Does dual targeting mitigate CD19 loss?

Number of tumors with CD19 and/or CD20 evaluation* at progression (N=27)



CD19/CD20

Only 1 patient experienced dual antigen loss

Conclusions from DALY II USA

Zamto-cel

- The first tandem CD20-CD19-directed non-cryopreserved CAR-T cell product
- Administered as a fresh product with a short vein-to-vein time of 14 days
- Lymphodepletion is initiated during the manufacturing process

KEY FINDINGS

- Pre-planned interim analysis of 59 evaluable patients - ORR 72.8%; CRR 50.8%
- 6-month PFS: 55% (95% CI: 41-67); median PFS: 9.0 months
- No grade ≥3 CRS
- Grade ≥3 ICANS in only 4.3% of patients
- Dual CD20-CD19 targeting appears to mitigate antigen loss as a mechanism of resistance
- No patient died while awaiting treatment with CAR-T

DALY II EU: Second Line RCT

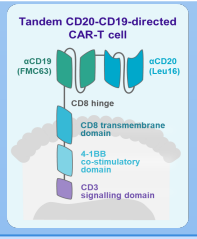
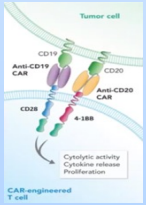
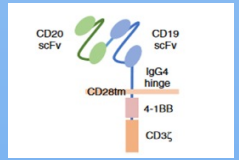
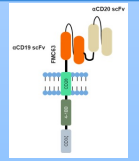
- Randomized Control Trial of second line R-Gem/Ox versus Zamtocabtagene Autoleucel in transplant ineligible population
- Will be presented as an Oral Presentation for the First Time at ASH 2025
- First randomized control trial in transplant ineligible patient population

Session Name: 628. Aggressive Lymphomas: Cellular Therapies: Novel Cellular Therapeutic Strategies for Aggressive Lymphomas
Session Date: December 7, 2025
Session Time: 4:30 PM - 6:00 PM
Presentation Time: 5:00 PM - 5:15 PM
Room: OCCC - Tangerine Ballroom F3-4

“Imitation is the sincerest form of flattery”

Explosion of 20.19 CAR T cells in DLBCL

2025: The year of Dual Targeted CARs

	Construct	Trials	NCT	Construct
MB20.19 CAR T cells (same construct as MCW LV20.19 CAR T cells)	Tandem 20.19 construct, 41BB	Phase II DALY US and EU trials - 3rd line DLBCL - 2nd line DLBCL transplant ineligible versus Gem/Ox	NCT04792489	
Kite-363 Product	Bicistronic CD20/41BB costim and CD19/CD28 costim	Phase 1 multi-center dose-escalation trial	NCT04989803	
Impact-Bio AKA Lyell	Tandem 20.19 construct, 41BB, enriched for CD62L+ naïve T cells	Phase II, multicenter study ongoing in aggressive DLBCL, CAR exposed and CAR naïve	NCT05826535	
Janssen	Tandem 20.19 construct (C-CAR039), 41BB	Phase 1B study, relapsed aggressive DLBCL	NCT05421663	

Updated Data Presented at Lugano

Product	Phase	N	ORR/CR	CRS/ICANS
Kite-363	1	26 patients at Dose Level 3	ORR 87%; CR rate 78%	Grade 3 ICANS 8% Grade 3 CRS 1 patient
JNJ CAR20.19	1	48 patients, multiple dose levels	ORR 90.5% and CR rate 76.2%	Grade 3 CRS 4% Grade 3 ICANS 6%
Lyell	1	45 CAR Naïve patients	ORR=94%, CR rate=74%	Grade 3 CRS=0 Grade 3 ICANS=13%
MB20.19	2	59 patients, 3rd line	ORR=71%, CR rate=55%	No Grade 3 CRS Grade 3 ICANS=3%

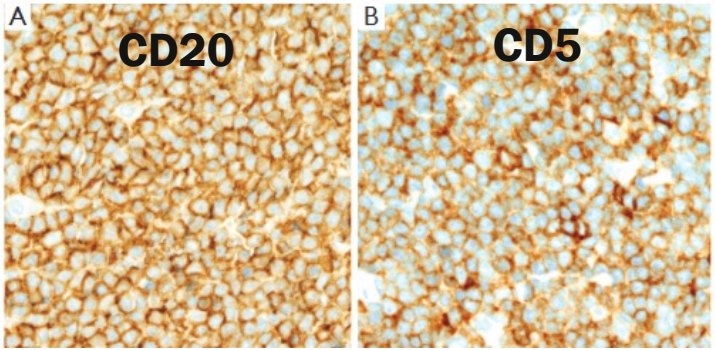
CD20.19 vs CD19

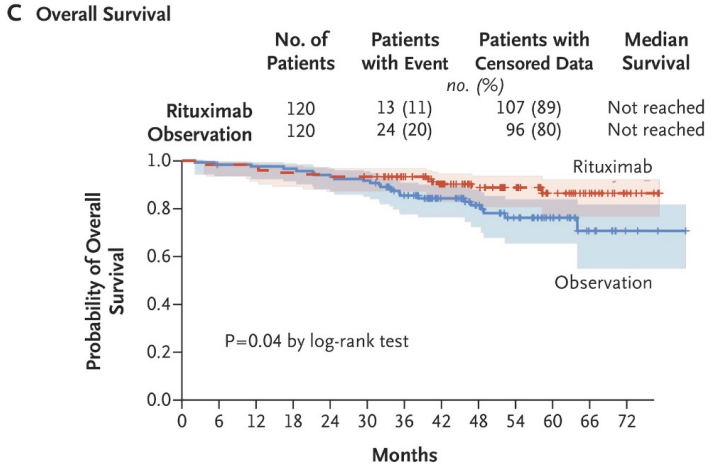
- Several companies are developing Dual Targeted CAR20.19 vs CAR19 phase III clinical trials
- Whether this will look better in a Phase III remains a major clinical question

Does Dual Targeting Improve Outcomes in non DLBCL histology?

Phase I/II MCL Arm

- Aggressive B-cell malignancy not felt to be curable with standard therapies
- Mature B-cell neoplasm that is defined by CD5 expression and high expression of CD20 with **survival benefit using antibodies that target CD20 (rituximab)**
- While approved CD19 CAR-T (Brexucabtagene autoleucel) is effective, relapses are common, and this therapy is limited by Grade 3+ CRS (15%) and Grade 3+ ICANS (31%)
- **Report final results from a single center Phase 1/2 trial of LV20.19 CAR T-cells in R/R MCL**





Journal of Clinical Oncology®
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ORIGINAL REPORTS | March 31, 2025

Phase I/II Study of Adaptive Manufactured Lentiviral Anti-CD20/Anti-CD19 Chimeric Antigen Receptor T Cells for Relapsed, Refractory Mantle Cell Lymphoma

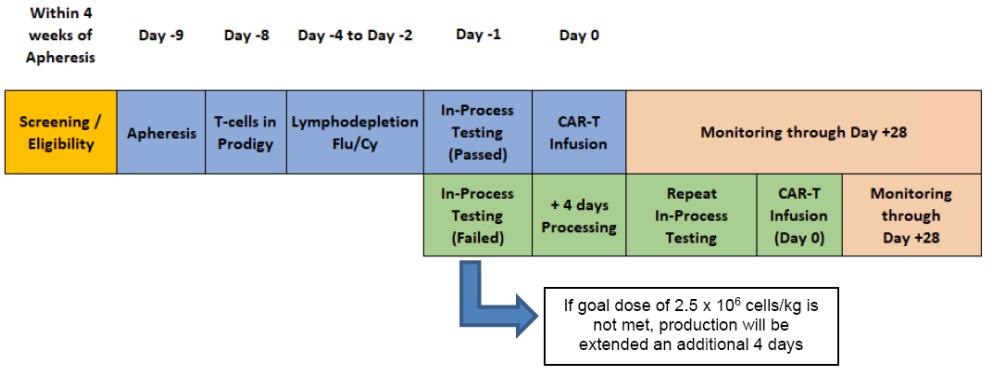
Authors: Nirav N. Shah, MD, MS, Alfredo S. Colina, BA, Bryon D. Johnson, PhD, Aniko Szabo, PhD, Fateeha Furgan, MD, Tyce Kearl, MD, PhD, Dina Schneider, PhD, Marlenny Vargas-Cortes, BS, Jessica L. Schmeling, BS, Michael B. Dwinell, PhD, Katie Palen, BS, Walter Longo, MD, Peiman Hematti, MD, Anthony E. Zamora, PhD, Parameswaran Hari, MD, MS, Daniel Bucklan, MD, Ashley Cunningham, MD, Mehdi Hamadani, MD, and Timothy S. Fenske, MD SHOW FEWER | AUTHORS INFO & AFFILIATIONS

Veloza, L., I. Ribera-Cortada, and E. Campo. *Annals of Lymphoma, 2019.*
Le Gouill et al. NEJM 2017

Trial Design

- Phase 1 safety run in followed by single stage Phase II design based on 3-month Ibrutinib CR rate of approximately 20%, target rate 50% (n=14)
- Key Inclusion: *Must have failed two lines of therapy or relapsed post autologous or allogeneic transplant*
- Utilized a flexible 8/12-day manufacturing platform with CliniMACS Prodigy® and goal of fresh infusion. Lymphodepletion started during CAR cell production.
- LDP Regimen: Fludarabine 30 mg/m2 + Cyclophosphamide 300 mg/m2 on Day -4, -3, -2

Population	r/r MCL
Design	Single-stage
Sample size	14
Endpoint	3 mo CR
P0 (P1) CR RATE	20% (50%)
Further develop if	6+/14
One-sided type I error	4.4%
Power	79%
Based on	Ibrutinib CR rate

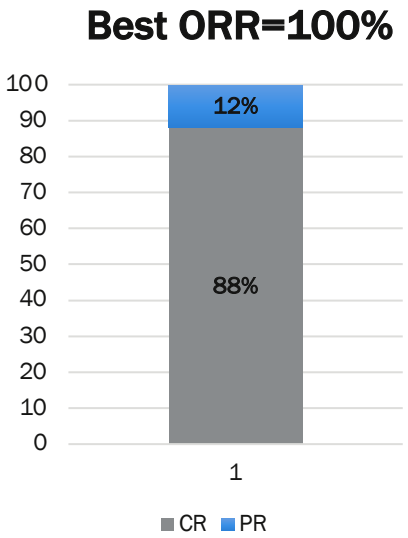


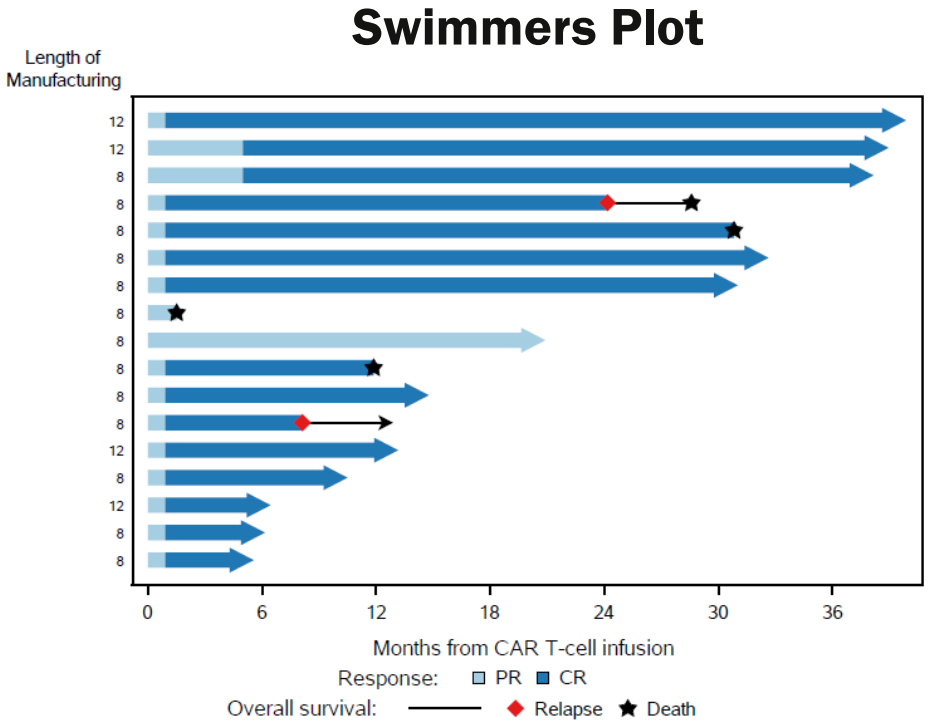
MCL Patients (Phase 1 + Phase 2)

- 4 patients received 12-day product
- 13 patients received 8-day product
- Manufacturing was successful in 100% of patients
- 3 patients received cryopreserved product
  - 1 patient with fungal sinus infection
  - 1 patient with rapidly progressive disease received R-CHOP after apheresis
  - 1 patient with COVID19 diagnosis delaying lymphodepletion start

MCL patients (n=17)	Phase 1=3 patients, Phase 2=14
Median Age, years	63 (range 50-74)
Male Sex	15 (88%)
Prior auto-HCT	8 (47%)
Prior allo-HCT	2 (12%)
LDH>Normal on Day 0	6 (35%)
BTKi exposed	16 (94%)
BTKi progressed	13 (76%)
Non-covalent BTKi progressed	6 (35%)
Prior Lines (including tx)	4 lines (range 2-8)
Prior Bendamustine	13 (76%)
Prior Bendamustine <1 year	2 (12%)
MIPI at Diagnosis (n=14) Low Intermediate High Missing	 6 patients 4 patients 4 patients 3 patients
Complex Cytogenetics	3 (18%)
p53 aberrations - p53 mutation - 17p deletion by FISH or cytogenetics	8 patients (47%) 6 patients (35%) 3 patients (18%)

Outcomes of LV20.19 CAR-T in R/R MCL

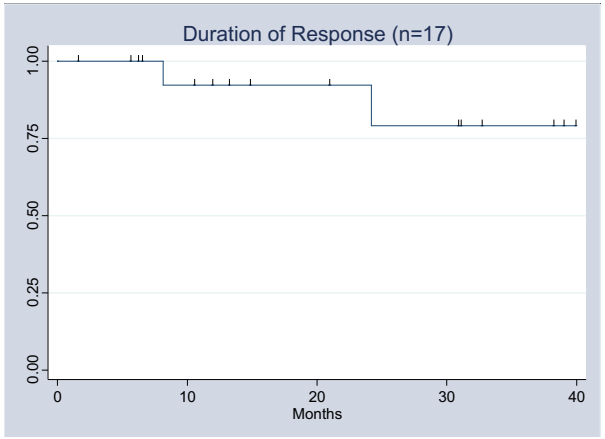




- **2 relapses to date**
- **Median follow-up 15 months**

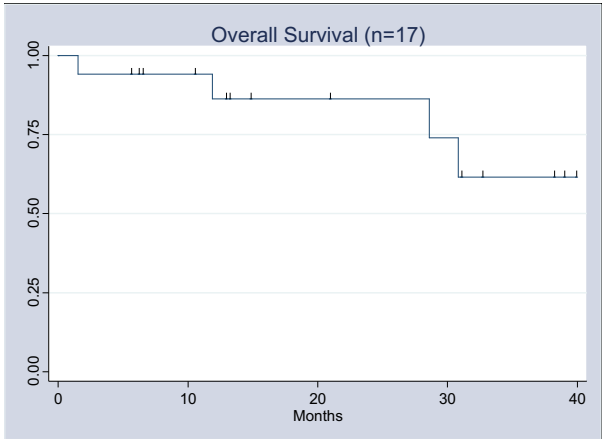
Phase II results, Expansion, and Survival Data

Day 90 CR rate for Phase II patients (n=14) was 86% (n=12) exceeding efficacy threshold. There is 1 PR at day 90 and 1 NRM (MRD-) that occurred prior to day 90.



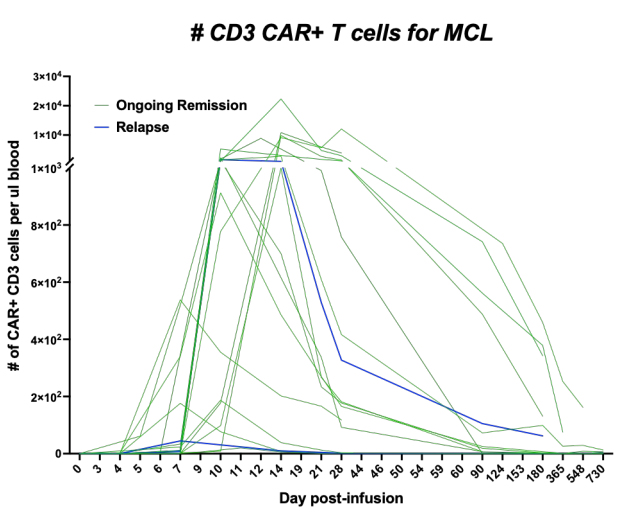
Duration of Response (above) (n=17)

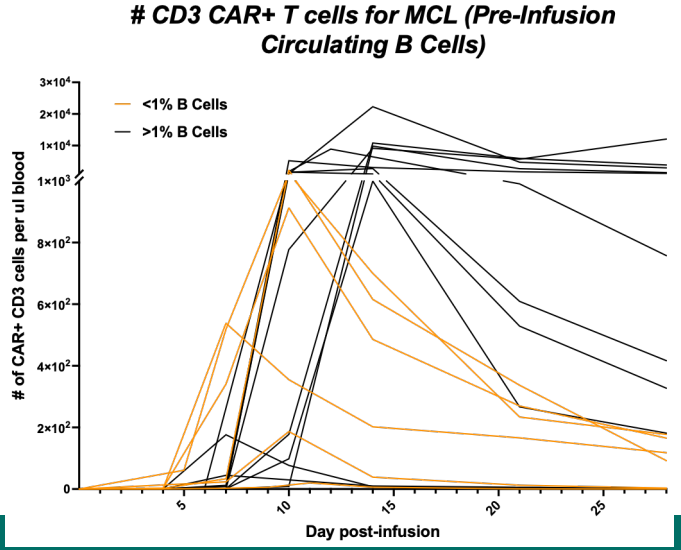
Overall Survival



In-vivo Expansion

- LV20.19 CAR T cells were detectable starting day +4 after infusion with peak expansion occurring between days 7-14 post infusion (left) with several patients with ongoing persistence >1 year post infusion
- Patients with >1% circulating B cells pre-infusion had increased CAR T cell expansion compared to patients with <1% circulating B cells pre-infusion (p=0.04) (right)



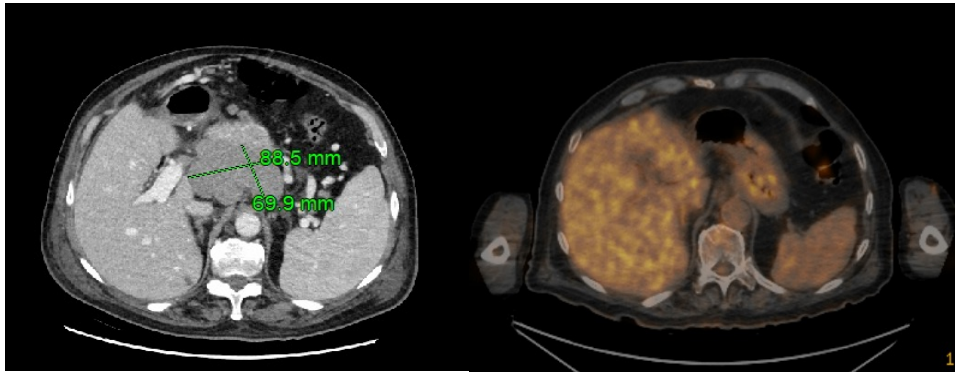


Imaging Response

Subject 40: 71-year-old male
P53 mutation, rapidly progressing MCL
Prior Lines: Nordic, Benda-Ritux, acalabrutinib, lenalidomide, CHOP, radiation
Response: Day 28 CR, ongoing +15 months

Pre-CAR CT scan
Bulky abd disease

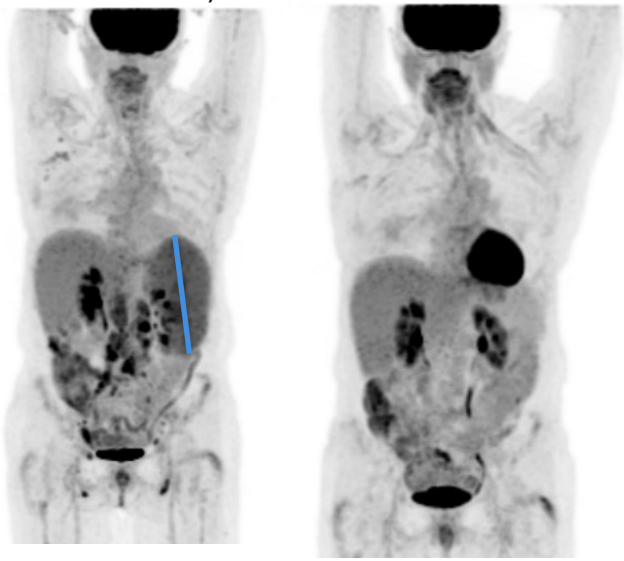
Day 28 PET/CT
Deauville 2



Subject 19: 63-year-old female
Prior lines: Hyper-CVAD, auto-HCT, Benda-Ritux, ibrutinib, velcade-rituximab, lenalidomide
Response: Day 28 CR, ongoing +31 months

Pre-CAR PET/CT

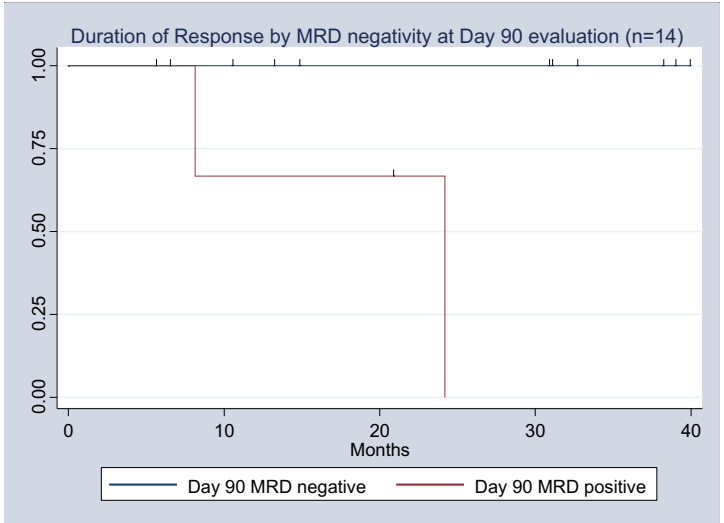
Day 28 PET/CT
Deauville 3



MRD Data

- Adaptive ClonoSEQ® MRD testing was utilized to assess patients
- Day 90 MRD negativity was predictive of relapse with no MRD negative patient at that time point relapsing to date ($p<0.01$)

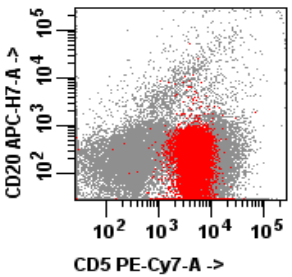
Adaptive ClonoSEQ MRD	MRD results
Initial MRD median 28 days (24-64 days) --- Negative --- Positive --- N/A	 12 pts (71%) 3 pts (18%) 2 pts (12%)
Day +90 MRD median 96 days (85-258 days) --- Negative --- Positive --- N/A	 11 pts (64%) 3 pts (18%) 3 pts (18%)

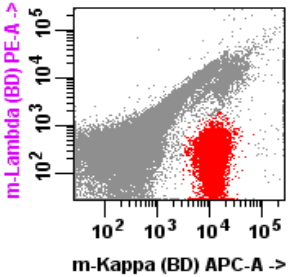


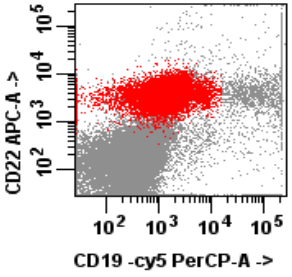
Relapsing Patients

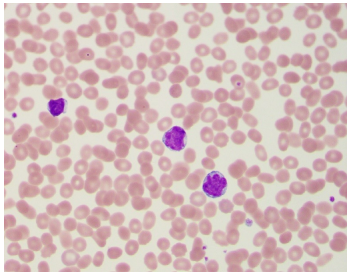
Subject 42

- Relapse +6 months after LV20.19 infusion
- Post-CAR relapse with high level of circulating CAR-Ts (15% of circulating CD3)
- CD20 negative, CD19+ (dim) at relapse
- Pre-CAR-T was CD20 negative, CD19 bright







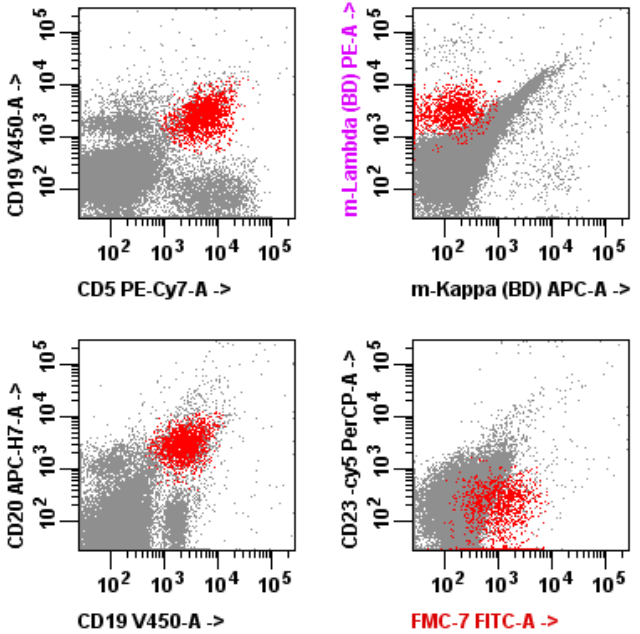


MoR: Antigen Downregulation

Relapsing Patients

Subject 12

- Relapse +2 years after LV20.19 infusion
- Post-CAR relapse with undetectable CAR-T cells
- Flow cytometry demonstrates no diminishment in CD19 and CD20 expression with pre-CAR biopsy.
- Pre-CAR biopsy: CD19 (87%) and CD20 (99%) and Post-CAR biopsy CD19 (100%) and CD20 (99%).



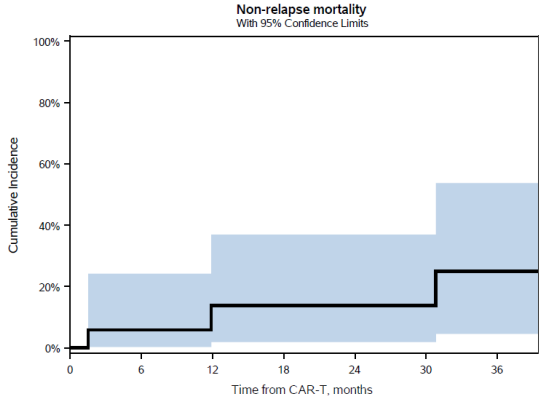
MoR: Loss of CAR persistence

Safety

Three non-relapse mortality events

+ 46 days: Gram negative Rod Sepsis
+ 12 months: COVID19 infection
+ 30 months: Viral infection followed by Guillen-barre

1-year non-relapse mortality rate of 13.8%

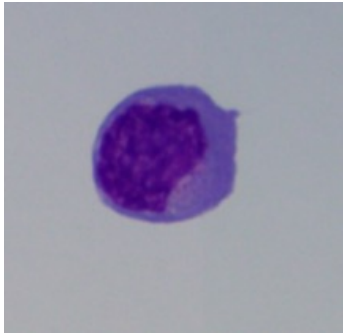


	N=17 patients
CRS Grade 1-2 Grade 3-4 ***No ICU level care in first 28-days*	16 pts (94%) 16 pts (94%) 0 pts
ICANS Grade 1-2 Grade 3-4	3 (18%) 1 (6%) 2 (12%)
IEC-HLH-like Syndrome - Defined as hyperferritinemia (>5000) + coagulopathy + LFT abnormalities	2 pts (12%)
Median ANC at Day 28 (K/μL)	1.71 (0.44-3.57)
Median ANC at Day 90 (K/μL)	1.71 (0.31-6.67)
Median Platelet at Day 28 (K/μL)	88 (18-202)
Median Platelet at Day 90 (K/μL)	175 (82-389)
Tocilizumab use	14 patients (82%)
Steroids administered	10 patients (59%)

Grade 3 ICANS

- Subject 42 developed Grade 2 ICANS on Day +8 started dexamethasone 10 mg q6hr
- Progressed to Grade 3 ICANS on Day +9.
- LP performed Day +9, given triple IT chemotherapy for ICANS, CSF incidentally found to be positive for MCL
- Repeat LP on Day +15 and Day +23 with no MCL and CAR T-cell expansion in the CSF
- CSF analysis demonstrates CAR-T engraftment with decreases in cytokines after IT administration

Day +9 CSF



CD5+ CD19+ MCL cells present
IT triple therapy administered

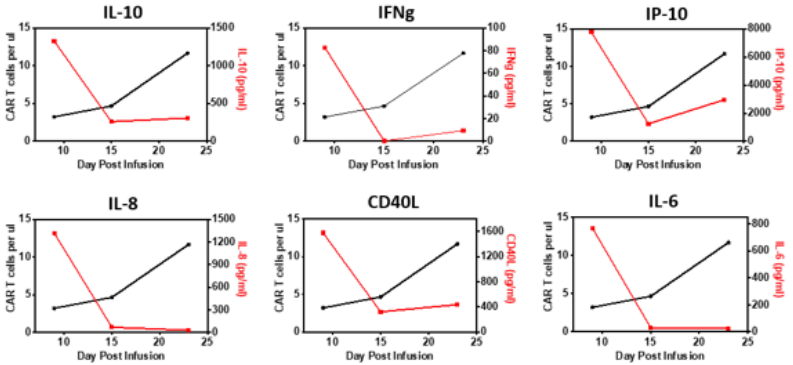
Day +15 CSF

No MCL
85% of CD3+ T-cells CAR+
Nucleated cells=6/uL

Day +23 CSF

No MCL
73% of CD3 T-cells CAR+
Nucleated cells=12/uL

Cytokine Analysis from CSF Subject 42 at 3 timepoints



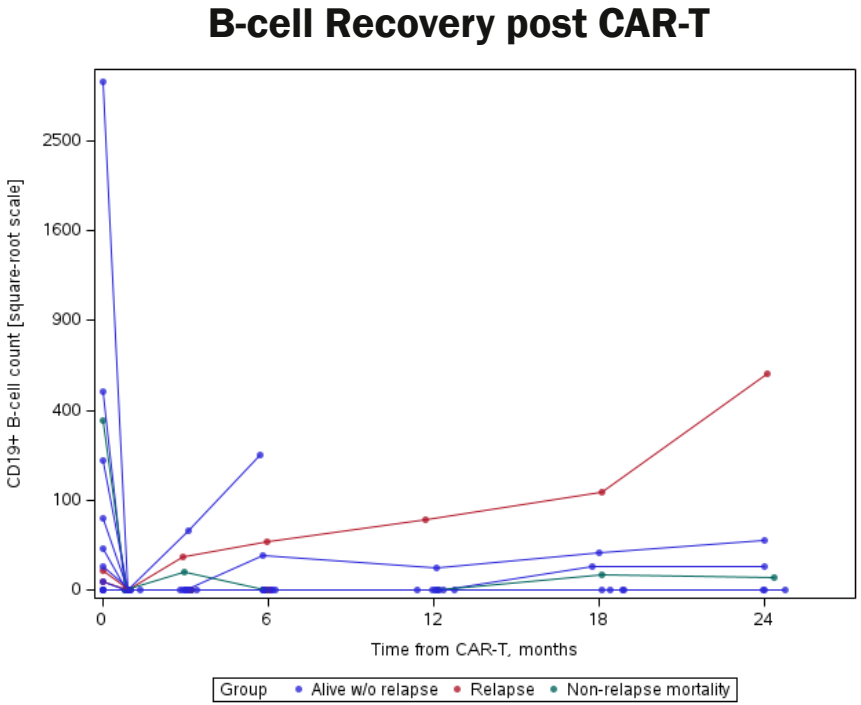
Neurotoxicity/ICANS

- ICANS (3 pts) within first 28 days
  - All managed with steroids + IT steroids/chemotherapy
  - 1 pt with Grade 3 ICANS had initial CSF positive for MCL that cleared on repeat CSF with engraftment of CAR + T-cells.
- Two Late Neurotoxicity events (>28 days): ICE 10/10, nonspecific confusion, numbness
  - Massive infiltration of CAR20.19 T cells in CSF (771 and 135 CAR+ cells/microliter)
  - Both treated with IT hydrocortisone alone (no systemic steroids) with resolution

CSF Samples

CSF Subject #	ICANS Max Grade	Day of CSF Analysis	Total Nucleated Cell Count	LV20.19 CAR+ T cells
20	Grade 3	Day +7	36/μL	15/μL
30	Grade 2	Day +12	41/μL	N/A
42	Grade 3	Day +9	12/μL (MCL present)	3/μL
		Day +15	6/μL	5 /μL
		Day +23	16/μL	11/μL
55	ICE 10/10, nonspecific neurotoxicity	Day +43	1005/μL	771/μL
57	ICE 10/10, nonspecific neurotoxicity	Day +60	386/μL	135/μL

Hematologic Recovery



- Median ANC at day 28 was 1.71 K/µL
- Median ANC at day 90 was 1.71 K/µL
- Median platelet count at day 28 was 88 and at day 90 was 175 K/µL
- Median IgG at day 90 was 398 (135-917) and 29% (n=5) of patients received IVIG supplementation post CAR-T.

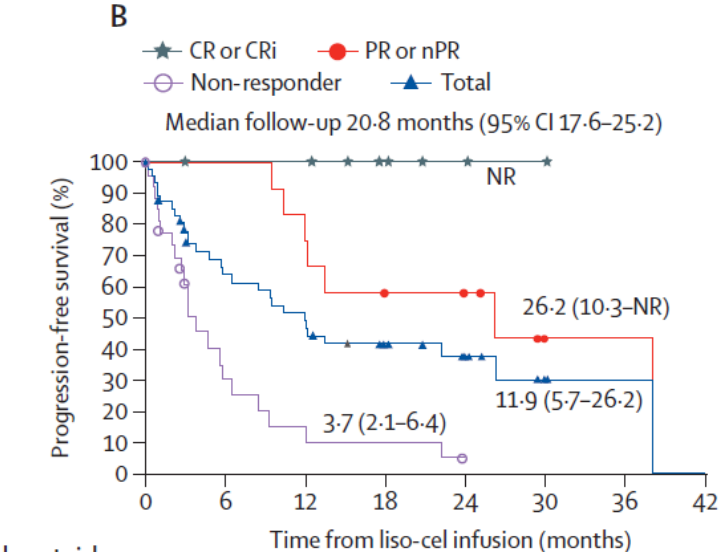
Cause of Death at Subject Level

Subject #	Time of Death Post CAR-T	Cause of Death
12	+29 months	Relapse
15	+31 months	meningoencephalitis
20	+1.5 months	Gram negative rod sepsis
31	+12 months	COVID19
42	+13 months	Relapse

- Two deaths were from relapse.
- Three patients had non-relapse mortality (NRM) events that included gram negative rod sepsis, COVID19 infection, and meningoencephalitis following a viral prodrome.
- All three NRM events occurred in the setting of ongoing B-cell aplasia (absolute B-cell<20/μL) at the time of death

Chronic Lymphocytic Leukemia

- While CLL is known to be a CD20 dim histology, targeting CD20 with antibodies has demonstrated benefit across clinical trials with improved benefit with higher affinity binding (e.g. benefit of Obinutuzumab over Rituximab).
- Largest trial of CD19 CAR-T in CLL with liso-cel demonstrated a median PFS of 11.9 months among 117 treated patients with higher toxicity than seen in other histologies (Grade 3+ CRS=9% and Grade 3+ ICANS=18%)
- Opened a single arm cohort of relapsed, refractory CLL



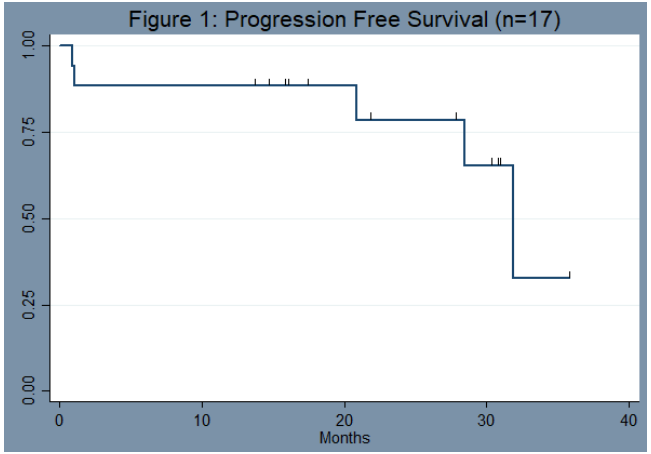
Siddiqi et al. Lancet 2023

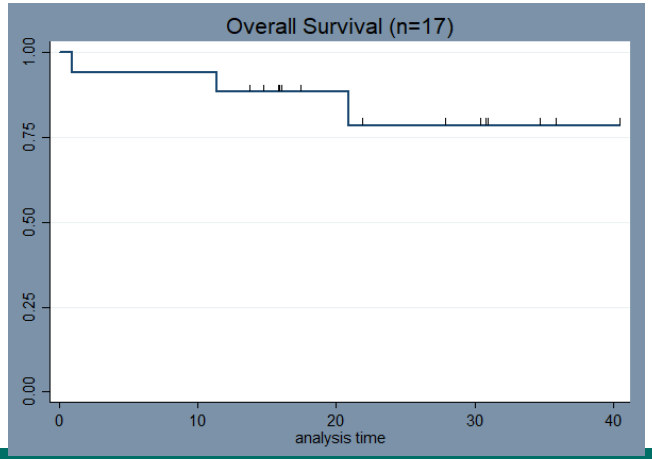
Brief Data Overview

- N=17 pts
- The median age was 65 years (34-75)
- Median prior lines was 3 (range 2-7).
- All patients had exposure to both BCL2i and covalent BTKi with 14 (82%) pts being refractory to both classes.

Response:

- Best ORR was CR/CRi in 14 (82%) patients.
- The median PFS for all pts was 32 months





CAR20.19 in Primary/Secondary CNS Lymphoma

MCW IIT Phase 1 Trial

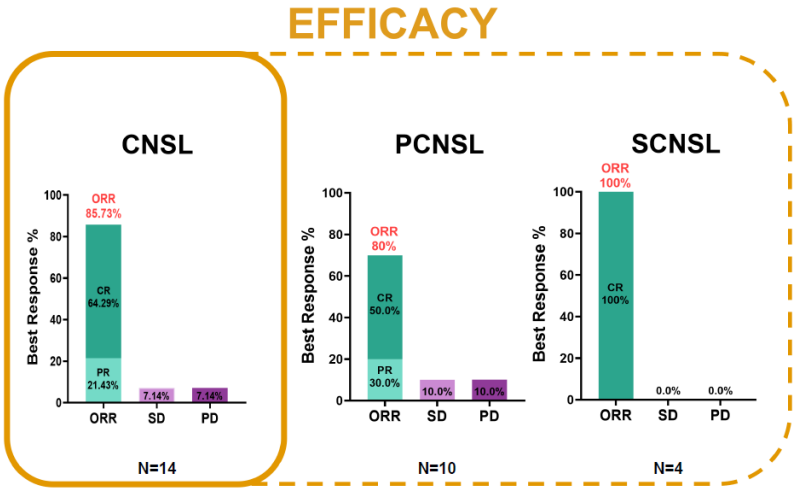
- Early data from our IIT (n=6), 100% ORR in patients with MTX relapsed/refractory primary and secondary CNS lymphoma
- High ORR but relapse remains a challenge
- Extended data will be presented at ASH by our fellow Dr. Ashley Dunton (details below)

ASH 2025

Details:
Session Name: 704. Cellular Immunotherapies: Early Phase Clinical Trials and Toxicities: Poster II
Session Date: December 7, 2025
Session Time: 6:00 PM - 8:00 PM
Presentation Time: 6:00 PM - 8:00 PM
Room: OCCC - West Halls B3-B4

Multicenter Effort in CNS Lymphoma

- Below is early data from Phase II trial of Zamtocabtagene CNS arms
- Early data, similar high ORR, limited follow-up to date



Johnston et al. Lugano 2025
Duntton et al. ASH 2025

Conclusions

1) Bispecific LV20.19 CAR T-cells are safe and efficacious across B-cell malignancies with a varying efficacy and toxicity profile. Multi-center phase II studies are ongoing t

2) All patients received CAR T-cells manufactured on-site utilizing a flexible 8-12 day platform with goal of fresh CAR T-cell infusion and lymphodepletion starting during manufacturing.

3) We have shown now across multiple studies, IT steroids/chemotherapy can be given to abrogate ICANS

4) Dual targeting may be more impactful in some histologies over others

Milwaukee to Dublin!

Acknowledgements

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Cell Therapy Lab
Bryon Johnson, PhD
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Tyce Kearl, MD, PhD
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